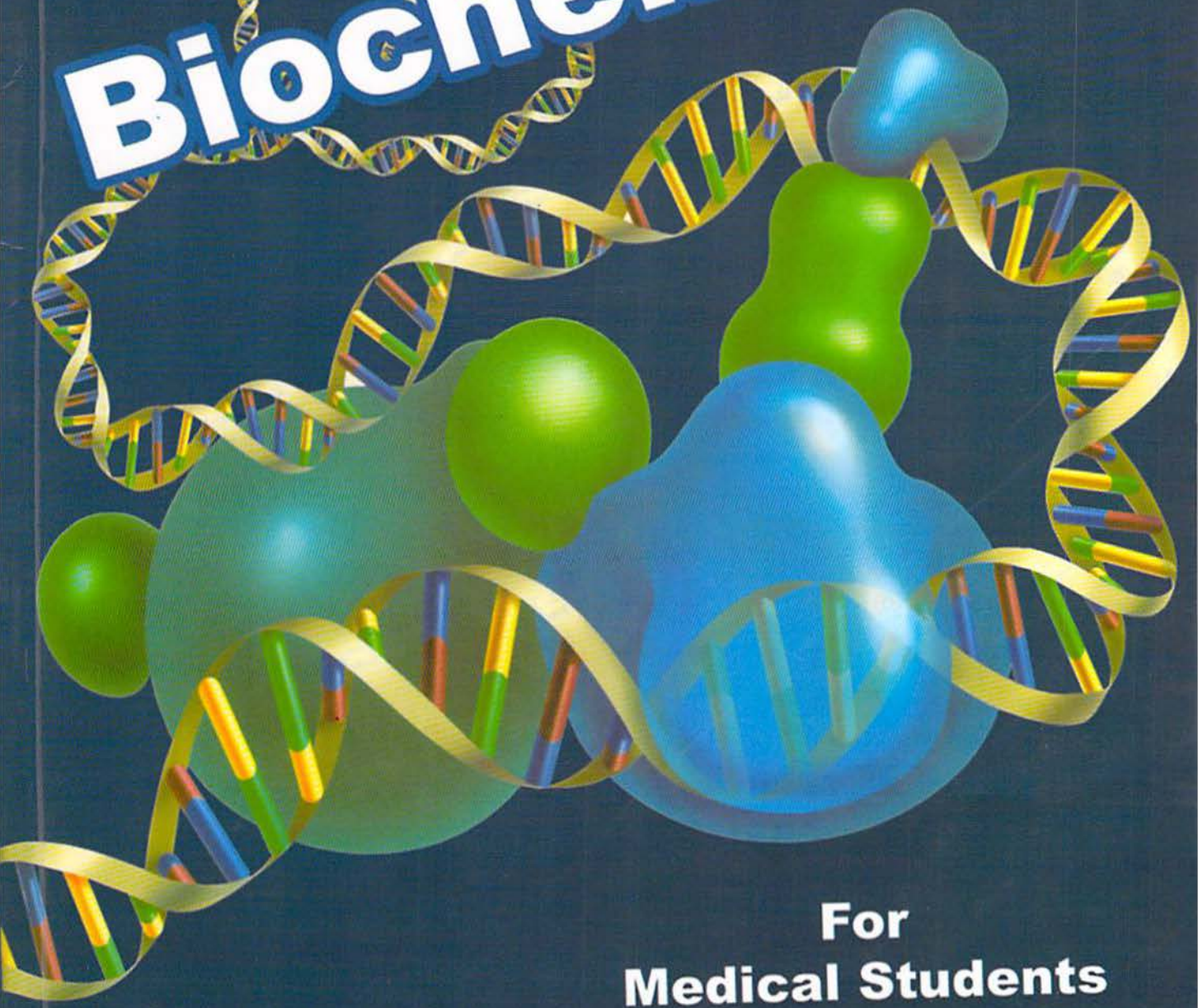
Oraby's Illustrated Reviews of Biochemistry



For
Medical Students
and Postgraduates
Part II

Oraby's Illustrated Reviews of

Biochemistry

For Medical Students
And Postgraduates

By

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(II)

Twelfth Edition

DEDICATION

To my wife

Note:

Dear student / colleague:

If you have any comment about this edition or further editions, please mail your suggestions to: m_s_oraby@hotmail.com.

Oraby's illustrated reviews of Biochemistry

First publication:	***1983***
2nd edition	***1984***
3rd edition	***1986***
4th edition	***1988***
5th edition	***1990***
6th edition	***1992***
7th edition	***1995***
8th edition	***1998***
9th edition	***2001***
10th edition	***2004***
11th edition	***2007***
Reprinted	*2008*
Reprinted	*2009*
12th edition	***2011***
Reprinted	*2013*

رقم الإيداع 2005 – 14489

الترقيم الدولي 7 – 368 – 224 - 977

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Chapter 1 — *Bioenergetics and Biologic oxidation*

Bioenergetics

I. *DEFINITIONS:*

A. Energy is the capacity to do work. It may be mechanical, chemical or electrical energy.

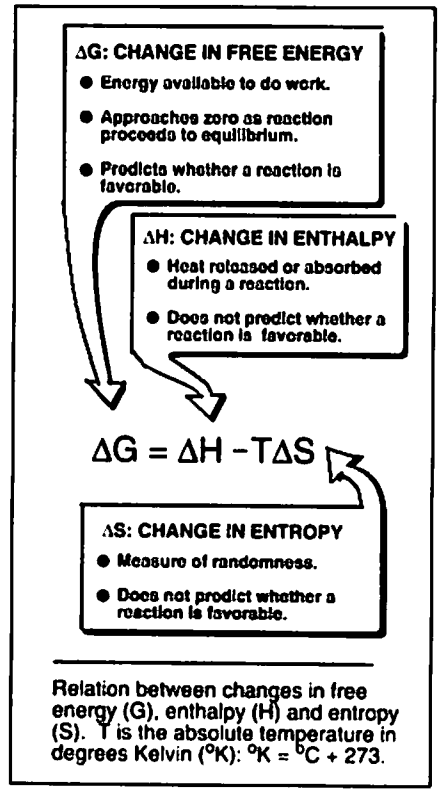
B. Bioenergetics or thermodynamics is the study of the energy changes accompanying biochemical reactions.

II. *TYPES OF ENERGY:*

A. **Heat energy:** is used to maintain body temperature.

B. **Free energy, = Delta G = (Δ G):**

1. Is useful energy for body activities as muscle contraction, nerve impulse etc.
2. The direction of chemical reaction is determined by 2 factors:
   a) Heat content (ΔH) of both reactant and product. It is called **enthalpy.**
   b) Randomness (Δ S) or disorder of both reactant and product. It is called: **entropy.**
   c) Combination of the 2 factors enthalpy (ΔH) - entropy (Δ S) will make a third factor called free energy (Δ G). So every substance has free energy, which determines the direction of reaction.



Relation between changes in free energy (G), enthalpy (H) and entropy (S). T is the absolute temperature in degrees Kelvin (°K): °K = °C + 273.

C. **<u>Forms of free energy (Free energy change, Δ G):</u>**

If we consider a reaction as follows: A ⇌ B

1. The direction of reaction may be A → B or B → A.
2. **Negative exergonic -ΔG: (A → B):** if ΔG is a negative number, this means that the free energy content of product (B) is less than that of product (A).

a) The reaction proceeds spontaneously in the cell from A →B. It is irreversible.

b) The reaction is said to be **exergonic.**

c) In these reactions there is a net loss of energy. This energy is used to form ATP. The compounds derived from food stuff intermediates (e.g. phosphoenol pyruvate, creatine phosphate etc) lose energy (~P) to form ATP.

d) Examples:

(i) Complete oxidation of creatine phosphate gives rise 10.3 Kcal/mol. In vitro this energy is lost in the form of heat. In vivo most of energy (**-ΔG 7** Kcal/mol is used to form ATP). The remaining energy (**-ΔG 3** Kcal/mol) is used to maintain body temperature.

Creatine~P + ADP → Creatine + ATP + **-ΔG 3** Kcal/mol

(ii) Complete oxidation of phosphoenol pyruvate gives rise 14.8 Kcal/mol. In vitro this energy is lost in the form of heat. In vivo most of energy (**-ΔG 7** Kcal/mol is used to form ATP). The remaining energy (**-ΔG 7.5** Kcal/mol) is used to maintain body temperature.

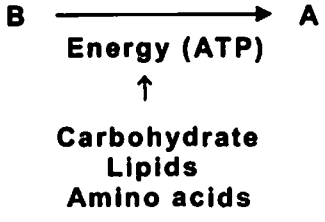
Phosphenol pyruvate + ADP → Pyruvate + ATP + **-ΔG 7.5** Kcal/mol

3. **Positive endergonic +ΔG:** (B → A) if ΔG is positive number, this means that the free energy content of (B) is more than that of product (A).

a) There is net gain of energy.

b) The reaction does not go spontaneously from B to A except after addition of energy (ATP) from another source. This source is the catabolism of carbohydrate, lipids (fatty acids) and amino acids.

c) The reaction is said to be **endergonic**



d) Examples:

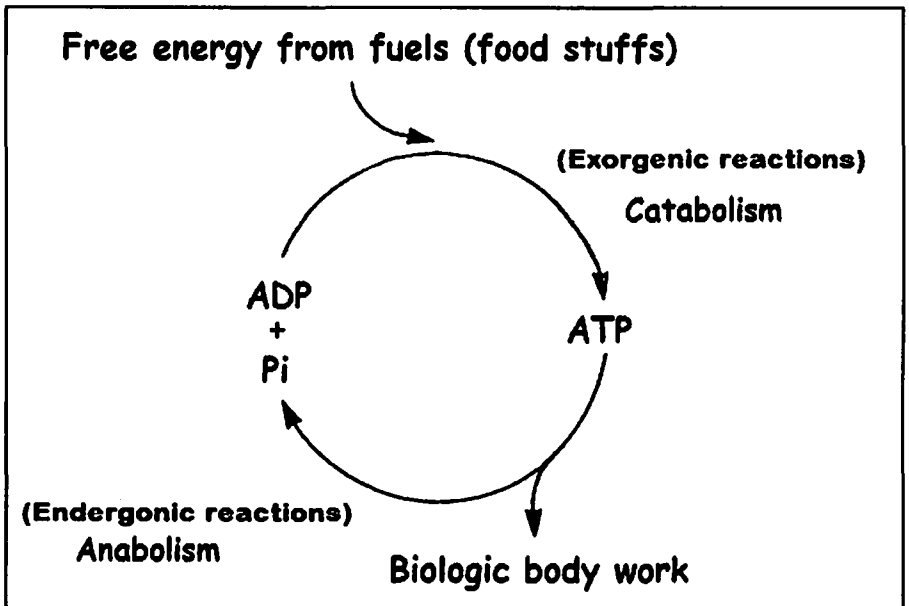
Creatine + ATP → Creatine~P + ADP + **+ΔG 1.5** Kcal/mol

4. **ΔG is zero:** if ΔG = 0, the reactants, are in equilibrium. [Note: when a reaction is proceeding spontaneously - that is free energy is being lost, then the reaction continues until ΔG reaches zero and equilibrium is established].
5. The **exergonic** reactions (energy producing reactions) are called: **catabolism** e.g. glycolysis and fatty acids oxidation. The **endergonic** reactions (energy utilizing reactions) are called: **anabolism** e.g. synthesis of glycogen and fatty acids. Both catabolism and anabolism constitute metabolism.

III. *ATP ACTS AS AN ENERGY CARRIER:*

A. Introduction:

1. The free energy made available through the catabolism of fuels (carbohydrates, lipids, and amino acids) is not transmitted directly to the reactions requiring energy. Instead it is used to synthesize a compound that acts as a carrier of free energy, which is adenosine triphosphate (ATP).
2. **ATP-ADP cycle:** It is the relationship between ATP generation and its utilization into ADP. ATP molecules are generated by exorgenic reactions. They are utilized (endergonic reactions) for different body works.

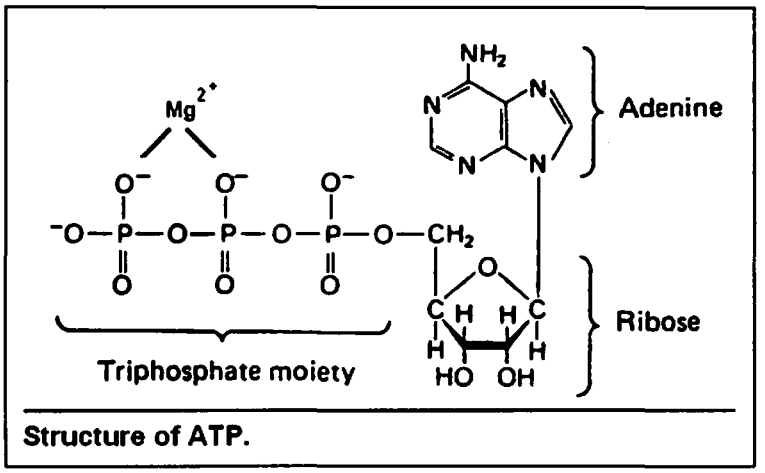


ATP is the link between energy-producing and energy-consuming biologic processes (ATP-ADP cycle). *Oraby*

B. Structure:

1. It is a member of a class of compounds called nucleotide triphosphates (ATP, GTP, UTP, CTP and TTP).

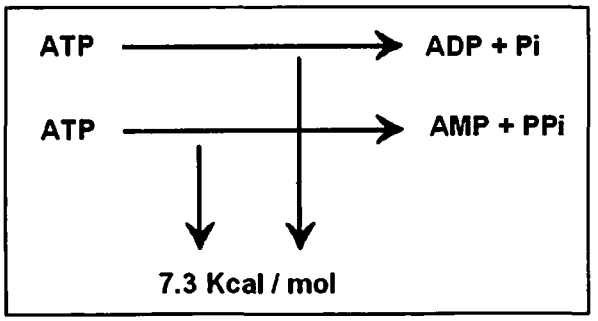
2. It has 3 component parts: a nitrogenous base (adenine), a sugar (ribose) and 3 phosphoryl groups that are joined to ribose by a phosphate ester bond and to each other by phospho-anhydride bonds.



Structure of ATP.

C. Importance of ATP:

1. The most important parts of the ATP molecule are its **2-phosphoanhydride bonds** (bonds between phosphate molecules number 1&2 and 2&3). Breakdown of either of these bonds is accompanied by a large decrease in free energy i.e. release energy.



2. Any bond whose breakdown is accompanied by a large decrease in free energy (≥ 5 Kcal/mol) is termed a **high energy bond** and represented by the symbol (~).
3. Breakdown of one high energy bond of ATP gives 7.3 Kcal.
4. Because ATP has two high energy bonds, it can serve as a link between energy yielding processes or **exergonic reactions** (e.g. catabolism of glucose and fatty acids) and energy requiring processes or **endergonic reactions** (e.g. anabolic pathways).
   a) Catabolic reactions give energy, which can be stored in the form of ATP.
   b) Anabolic reactions can utilize energy through hydrolysis of ATP.

IV. *UNITS OF FREE ENERGY:*

A. **Traditional units *(calorie)*:** energy measured in calories (one calorie is the amount of energy required to raise one gram of water by one degree (15°C-16°C).

B. **SI units *(joule)*:** energy measured in joules (one calorie = 4.128 joule).

V. *CALCULATION OF CALORIES:*

By using Bomb calorimeter (complete oxidation of foodstuff by burning in the presence of oxygen).

A. One gram of carbohydrate → 4 Kcal

B. One gram of fat → 9 Kcal

C. One gram of protein → 41 Kcal.

VI. *DIFFERENCE BETWEEN IN VITRO AND IN VIVO OXIDATION OF FOODSTUFF:*

A. **In vitro:** all free energy is released as heat.

B. **In vivo:** part is conserved in the form ATP and the remaining is released as heat.

VII. *PHOSPHATE BONDS:*

A. **High energy phosphate bonds:** Give rise to 7.3 Kcal/mol. Examples:

1. **ATP:** Its free energy is about -7.3Kcal/mol **(see before).**
2. **Creatine phosphate (C~P):** Excess ATP is stored in the form of C~P in muscles. Its free energy is about -10.3 Kcal/mol. (see protein metabolism)
3. **Carbomyl phosphate:** Its free energy is about -12.3 Kcal/mol. (see protein metabolism)

B. **Low energy phosphate bonds:** Give rise **less than** 7.3 Kcal/mol. Examples:

1. **Glucose-1-phosphate:** -5 Kcal/mol.
2. **Glucose-6-phosphate:** -3.3 Kcal/mol.
3. **Fructose-6-phosphate:** -3.8 Kcal/mol.
4. **AMP:** -3.4 Kcal/mol.
5. **Glyceraldehyde-3-phosphate:** -2.2 Kcal/mol.

Biologic oxidation

I. *Introduction:*

A. Energy is required to maintain the structure and function of the living Cells. This energy is derived from oxidation of carbohydrates, lipids and proteins of diet.

B. The energy liberated is partly converted into useful form ATP, which is known as ***energy currency*** *of the living cells.*

C. Each gram of carbohydrate and protein gives about 4 Kcal on oxidation, while each gram of fat gives about 9 Kcal.

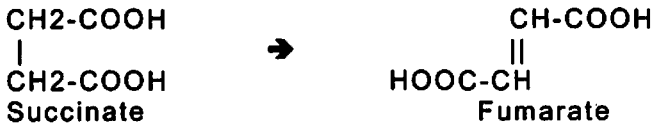
II. *Oxidation and reduction:*

A. **Oxidation means:**

1. **Addition of oxygen to a compound:**

$$2\ Cu + O_2 \rightarrow 2\ CuO \text{ (cupric oxide).}$$

2. **Removal of hydrogen from a compound:**



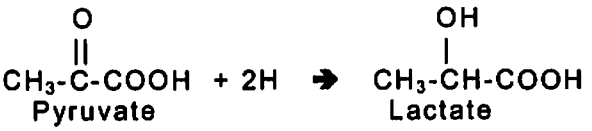
3. **Removal of electron from an ion or atom** i.e. increases in positive charges of an atom or an ion:

$$Fe^{++} \rightarrow Fe^{+++} + e$$

B. **Reduction means:**

1. **Removal of oxygen from a compound.**

2. **Addition of hydrogen to a compound:**



3. **Addition of electron to an ion or atom** i.e. decrease in positive charges of an atom or an ion:

$$Fe^{+++} + e \rightarrow Fe^{++}$$

C. Commonly the oxidation reactions are accompanied by reduction reactions and they are called **redox reactions**. Redox reactions are accompanied by energy liberation, necessary for the cells.

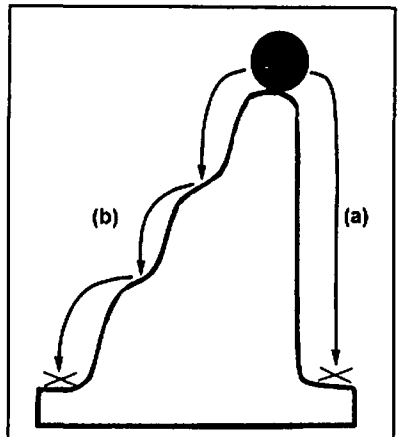
1. **Hydrogen** plays an important role in liberation of energy as represented by the following example:

$$2\ H_2 + O_2 \rightarrow 2\ H_2O + \text{energy}$$

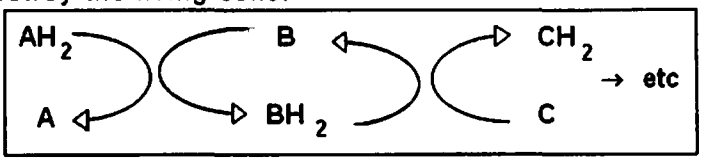
2. In this redox reaction, H_2 is oxidized while O_2 is reduced, and if occurs it will be accompanied by a massive energy explosion.
3. This simple example is similar to the fundamental reactions which provide energy in the living cells, and instead of massive energy is liberated, hydrogen must be transferred to oxygen in gradual steps. Thus, small fractions of energy are liberated and stored for further use.
4. Note that hydrogen atom is formed of one electron (e) and one proton (H^+). The removal of hydrogen atom or electron from a compound is always accompanied by a release of energy.

C. **Redox potential (= electron affinity):**

1. Oxygen has the highest electron affinity i.e. highest redox potential.
2. Hydrogen has the lowest electron affinity i.e. lowest redox potential.
3. **Redox chain:**
   a) **It is a chain of different compounds of increasing redox potentials between hydrogen and oxygen**
   b) **The living cells depend on redox reactions for their energy requirements. The reactions start by removal of H_2 from the substrate, which is then transferred to different components of redox chain, and finally to oxygen to form water. Components of redox chains have a redox potential higher than hydrogen and lower than oxygen.**
   c) During hydrogen (H^+ and electron) transfer through different components of the redox chain, energy is liberated in steps and in small utilizable amounts instead of a massive energy production, in the form of heat, which if happens may destroy the living cells.



Model of energy loss by big rock rolling down hill using two different pathways:
a. Direct with massive energy production.
b. Indirect in steps with release of small amounts of energy.



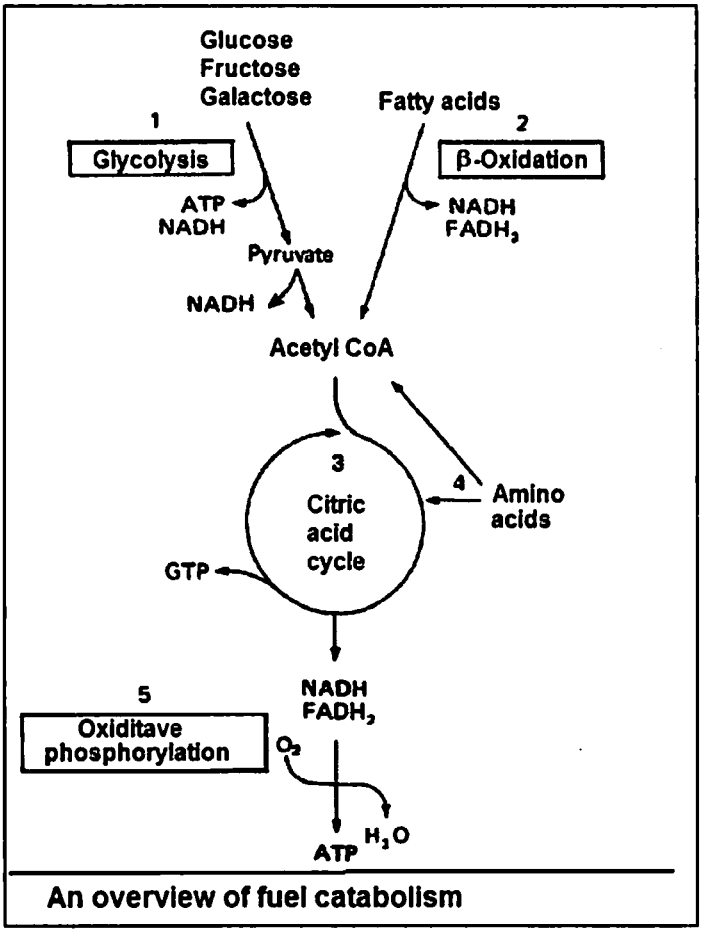
Hydrogen is transferred from A to B and from B to C because B has a higher redox potential (electron affinity) than A, while C has a higher redox potential than B... etc. In this way hydrogen (electron + H^+) moves from compounds of lower redox potential to compounds of higher redox potential.

III. *Respiratory chain (also called electron transport chain):*

A. Definition:

It is the final common pathway in aerobic cells by which electrons derived from various substances are transferred to oxygen to form water.

1. A variety of substances (carbohydrate, amino acids and fatty acids) can use respiratory chain as a final pathway as they give electrons to oxidized coenzymes NAD^+ and FAD^+ to form the energy rich reduced coenzymes, $NADH^+$, $FADH_2$.



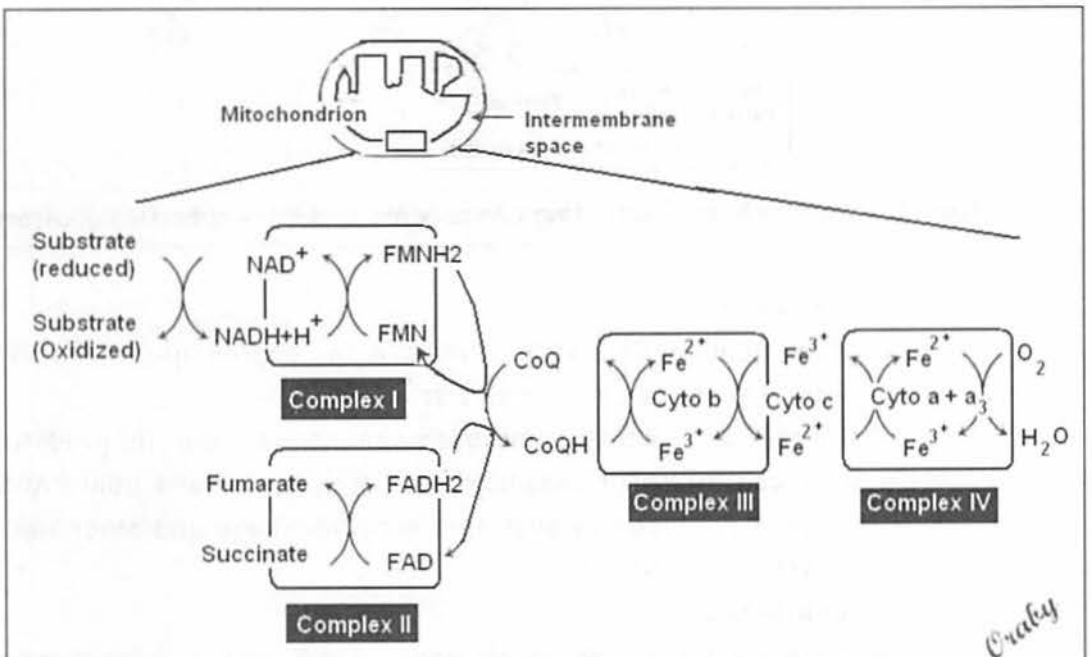
An overview of fuel catabolism

2. $NADH^+$, $FADH_2$ give hydrogen and a pair of electrons to electron carriers collectively called the **respiratory chain components**.
3. Hydrogen and electrons flow through respiratory chain in steps from the more electronegative component (low redox potential) to the more electropositive substance. **Oxygen** is the most electropositive component i.e. has the highest electron affinity (=highest redox potential). So oxygen is the final acceptor of electrons and protons in respiratory chain.
4. As electrons are passed down the respiratory chain, they lose much of their free energy. Part of this energy can be captured

and stored by the production of ATP from ADP and inorganic phosphate(Pi). This process is called **oxidative phosphorylation**. The remainder of the free energy not trapped as ATP is released as heat.

B. **Organization of the respiratory chain :**

1. The inner mitochondrial membrane contains 5 separate enzyme complexes, called complex I, II, III, IV and V. Complex V catalyses ATP synthesis.
   a) **Each complex** accepts or donates electrons to relatively mobile electron carriers such as coenzyme Q and cytochrome C.
   b) **Each carrier** of electron transport chain can receive electrons from the more electronegative donor and can subsequently donate electrons to the next more electropositive carrier in the chain. Finally electrons combine with oxygen and protons to form water.



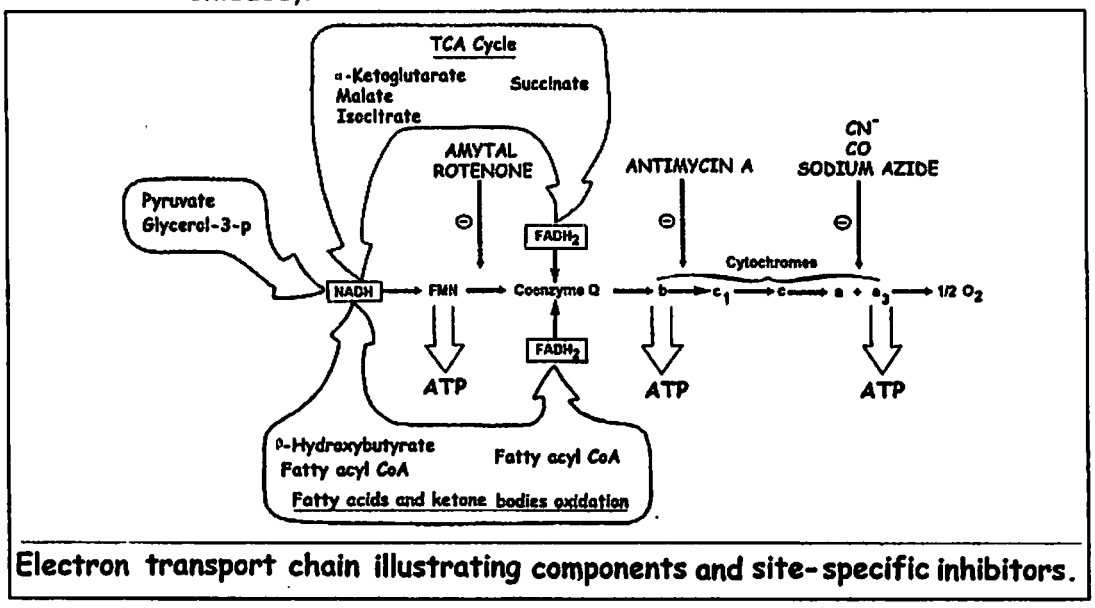
Electron transport chain. [Note: Coplex V not shown].

2. **Components of the respiratory chain:** All members of the respiratory chain are protein except coenzyme Q. All are embedded in the inner mitochondrial membrane.
   a) **Complex I:** Contains an enzyme called NADH dehydrogenase
      (i) Its coenzyme is FMN.
      (ii) It contains several iron and sulfur atoms.
      (iii) It oxidizes $NADH+H^+$ into NAD. At the same time converts its coenzyme FMN into $FMNH_2$.

b) **Complex II:** Contains an enzyme called: flavoprotein dehydrogenase e.g. succinate dehydrogenase of TCA and acyl CoA dehydrogenase of fatty acid oxidation.
   (i) Its coenzyme is FAD.
   (ii) It contains iron and sulfur atoms.

c) **Complex III**: contains cytochrome b enzyme and cytochrome c_1.

d) **Complex IV**: contains cytochromes a + a_3 (=cytochrome c oxidase).



Electron transport chain illustrating components and site-specific inhibitors.

3. **Coenzyme Q:**
   a) It is quinine derivative with a long isoprenoid tail. It is a relatively mobile electron carrier.
   b) Coenzyme Q can accept hydrogen atoms both from $FMNH_2$, produced by NADH dehydrogenase (complex I) and from $FADH_2$, which is produced by succinate dehydrogenase and other similar enzymes (complex II).
4. **Cytochromes:**.
   a) There are 5 types of cytochromes; cyto b, cyto c_1, cyto c, cyto a and cyto a_3.
   b) All cytochromes are conjugated proteins formed of protein conjugated with heme ring. The heme ring contains iron (Fe). This iron oscillates between ferric ions (Fe^{3+}) when it loses an electron, and ferrous (Fe^{2+}) when it accepts electrons.
   c) Cytochrome b is associated with sulfur (S) in addition to iron (Fe).

d) **Cytochrome c:** is a relatively mobile carrier. It receives electrons from complex III (cyto b and cyto c_1) and transfer them to cytochrome c oxidase (cyto a and cyto a_3).

e) **Cytochrome c oxidase (a and a_3):** it is a complex that contains two heme, cytochrome a, cytochrome a_3, iron and copper. It is the last enzyme in the respiratory chain. It is the only electron carrier that can react directly with molecular oxygen. It receives electrons from cyto c and transfer them to one oxygen molecule.

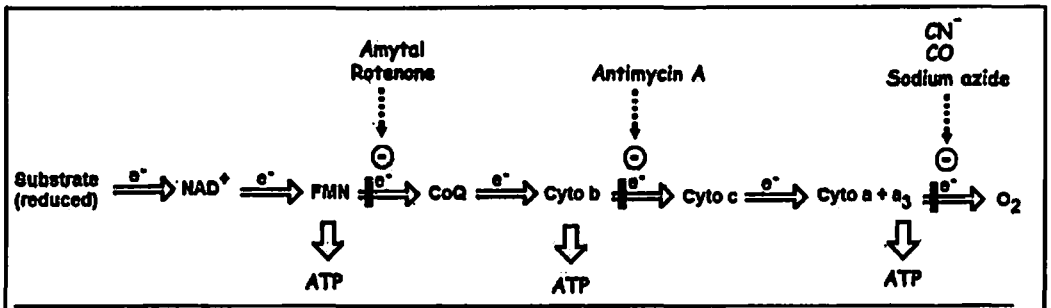
C. Reactions of respiratory chain:

1. **Entry via NADH + H^+:** NADH + H^+ obtained from reactions catalyzed by dehydrogenase enzymes e.g. dehydrogenase of TCA can join the chain giving electrons to FMN of complex I to coenzyme Q to cytochrome b, cytochrome c to cytochrome a + a_3 to the final acceptor O_2.
2. **Entry via $FADH_2$:** $FADH_2$ obtained from reactions catalyzed by flavoprotein dehydrogenase e.g. succinate dehydrogenase can join the chain directly giving electrons to coenzyme Q, then to cytochrome b, c, a + a_3 to the final acceptor oxygen.

D. Inhibitors of respiratory chain:

Are compounds preventing the passage of electrons by binding to a component of the chain, blocking the oxidation - reduction reaction.

1. There are specific sites for binding inhibitors.
   a) **Site I:** binding with complex I, preventing passage of electrons from FMN to coenzyme Q.
      (i) Example of inhibitors: **Barbiturates, "piericidin A"** antibiotic and by the insecticide and fish poison"rotenone".
   b) **Site II:** binding with complex III, preventing passage of electrons from cytochrome b to cytochrome c.
      (i) Example: Antimycin A and dimercaprol.
   c) **Site III:** binding with complex IV, preventing passage of electrons from cytochrome a + a_3 to O_2.
      (i) Example of inhibitors: H_2S, cyanide (CN^-), carbon monoxide and sodium azide.

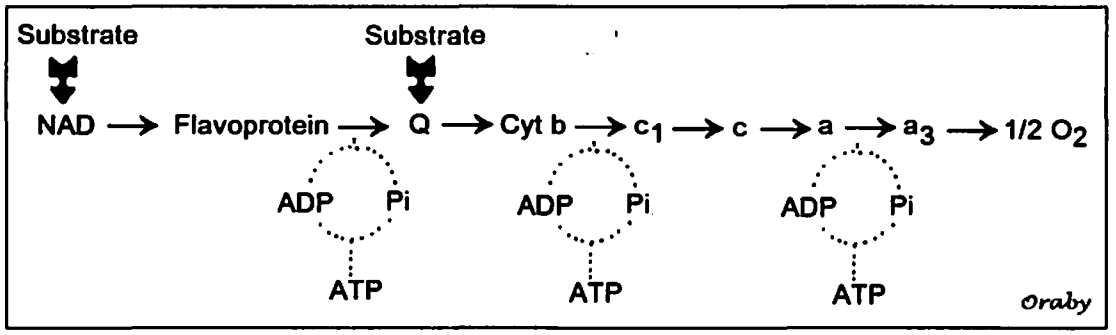


Site of respiratory chain inhibitors.

2. Because electron transport and oxidative phosphorylation are tightly coupled, inhibition of the respiratory chain also inhibits ATP synthase.

E. Release of free energy during electron transport:

1. **Free energy** is released as electrons are transferred along the electron transport chain from electron donor (reducing agent or reductant) to an electron acceptor (oxidizing agent or oxidant).
2. The electrons can be transferred in different forms, for example:
   a) As hydride ion (H) to NAD^+.
   b) As hydrogen atoms (H) to FAD.
   c) As electrons (e) to cytochromes.
3. **Redox pairs** (NAD^+ / $NADH+H^+$), (FAD / $FADH_2$) differ in their tendency to lose electrons. Each redox pair has a characteristic tendency (E_o) and it is constant to that pair. Its units are measured in volts. E_o is called standard reduction potential.
4. **E_o (standard reduction potential):** The standard reduction potentials of various redox pairs can be listed to range from the most negative E_o **to the most positive one.**
   a) The more negative E_o of a redox pair, the greater the tendency of that pair to lose electrons.
   b) The more positive E_o, the greater the tendency of that pair to accept electrons.
   c) Therefore, electrons flow from the pair with the more negative E_o to that with the more positive E_o.
5. **At three sites** (see the figure), the free energy released per electron pair transferred is sufficient to support the phosphorylation of ADP to ATP, which required about 7 Kcal/mol.



6. Electrons that enter the respiratory chain through the NAD-Q reductase complex support the synthesis of 3 mol of ATP. By contrast, electrons join the chain directly at the level of coenzyme Q (as in case of $FADH_2$ of succinate

dehydrogenase) will only support the synthesis of 2 mol of ATP.

Oxidation-reduction potentials and free-energy changes at sites in the electron transport chain that can support ATP formation:

Site	Oxidation-reduction potential E_0 (volts)	Free energy released (Kcal/mol)
At NAD^+ - Q	0.27	12.2
At cytochrome b →cytochrome c_1	0.22	09.9
At cytochrome a_3 → O_2	0.53	23.8

7. **P/O ratio:** It is the ratio between numbers of ADP mol changed into ATP to the number of oxygen atom (1/2 O_2) utilized by respiratory chain. It is 3:1 if electrons enter through NAD-coenzyme Q and it is 2:1 if electrons join the chain directly at the level of coenzyme Q.

d) *Oxidative phosphorylation:*

A. Introduction:

1. **Electrons are transferred down the respiratory chain from $NADH^+$ to oxygen. This is because $NADH^+$ is a strong electron donor, while oxygen is a strong electron acceptor.**
2. **The flow of electrons from $NADH^+$ to oxygen (oxidation) results in ATP synthesis by phosphorylation of ADP by inorganic phosphate, Pi (phosphorylation). Therefore, there is a coupling between oxidation and phosphorylation. Two theories explain the ATP synthesis, chemiosmotic hypothesis and membrane transport system.**

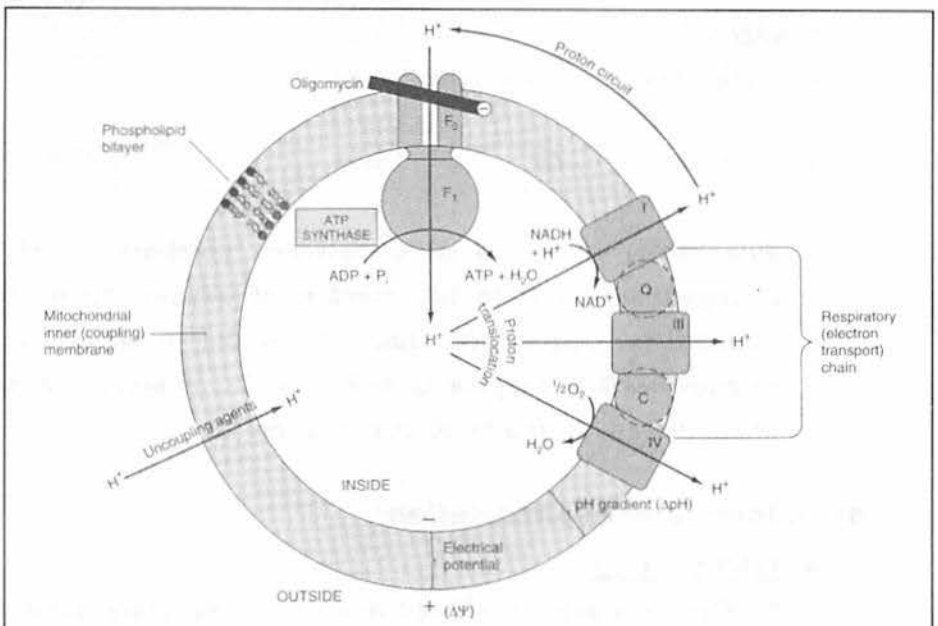
B. Chemiosmotic hypothesis:

Also called Mitchell hypothesis. This hypothesis is one form of oxidative phosphorylation. It can be summarized as follows:

1. **Proton pump:**
   a. **The transport of electrons down the respiratory chain → Gives energy.**
   b. **This energy is used to transport H+ from the mitochondrial matrix → from inside to outside the membrane.**
   c. **This is done by complexes I, III and IV.**
   d. **This process creates across the inner mitochondrial membrane:**
      i. **An electrical gradient: (with more positive charges on the outside of the membrane than on the inside).**

ii. **A pH gradient**: (the outside of the membrane is at lower pH than the inside).

e. The energy generated by this proton gradient is sufficient for ATP synthesis.



Principles of the chemiosmotic theory of oxidative phosphorylation. The main proton circuit is created by the coupling of oxidation in the respiratory chain to proton translocation from the inside to the outside of the membrane, driven by the respiratory chain complexes I, III, and IV, each of which acts as a *proton pump*. Q, ubiquinone; C, cytochrome *c*; F_1, F_0, protein subunits which utilize energy from the proton gradient to promote phosphorylation. Uncoupling agents such as dinitrophenol allow leakage of H^+ across the membrane, thus collapsing the electrochemical proton gradient. Oligomycin specifically blocks conduction of H^+ through F_0.

2. **ATP synthase (complex V):**

   In the inner mitochondrial membrane, there is a phosphorylating enzyme complex: **ATP synthase** (or complex V).

   a) It is formed of 2 subunits:

      (i) F_1 subunit which protrudes into the matrix.

      (ii) F_o subunit which is present in the membrane.

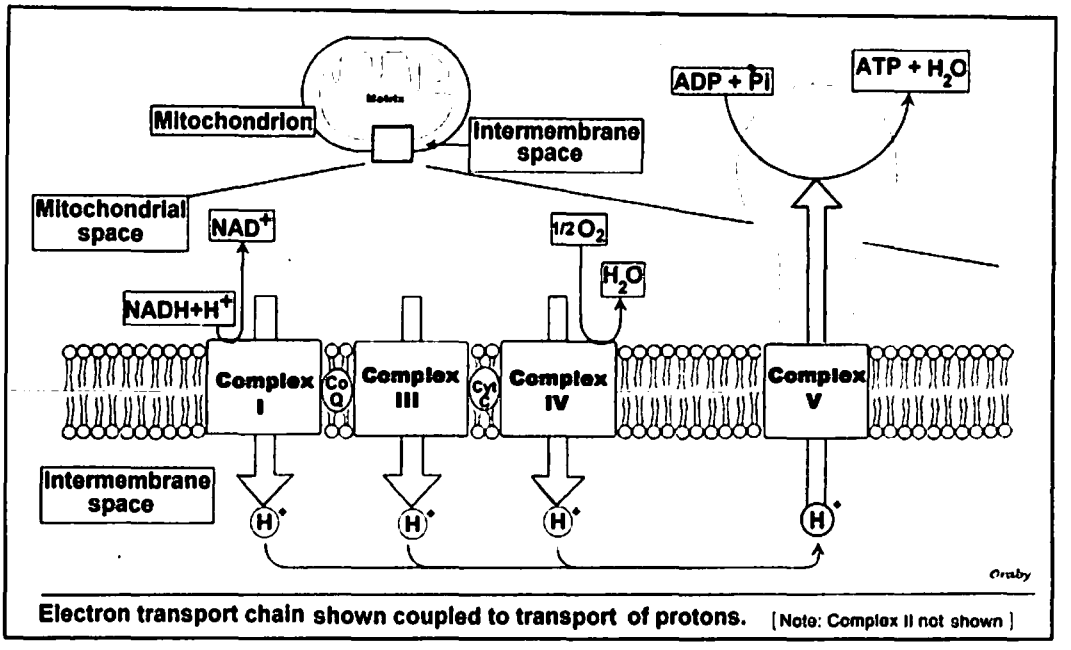
   b) The protons outside the inner mitochondrial membrane can re-enter the mitochondrial matrix by passing through channel (F_o- F_1 complex) to pass by ATP synthase enzyme which is present in F_1 subunit. This results in the synthesis of ATP from ADP + Pi. At the same time decrease the pH and electrical gradients.

3. **Evidences support chemiosmotic theory:**

   a) Addition of protons (acid) to the external medium of intact mitochondria leads to the generation of ATP.

   b) ATP synthesis does not occur in soluble cytosol system where there is no ATP synthase. A closed membrane as mitochondria must be present in order to obtain oxidative phosphorylation.

c) The component of respiratory chain is organized in a sided manner as required by chemiosmotic theory.



Electron transport chain shown coupled to transport of protons. [Note: Complex II not shown]

4. **Uncouplers:**

These are substances that allow oxidation to proceed but prevent phosphorylation. So energy released by electron transport will be lost in the form of heat. This explains the cause of hotness after intake of these substances. Examples:

a) **Oligomycin:** This drug binds to the stalk of the ATP synthase , closes the H^+ channel, and prevent re-entry of protons to the mitochondrial matrix.

b) **2,4 Dinitrophenol:** It increases the permeability of the inner mitochondrial membrane to proton causing decrease proton gradient.

c) **Calcium and high doses of aspirin:** this explains the fever that accompanies toxic overdoses of these drugs.

d) **Ionophores:** e.g. antibiotics "valinomycin" and Nigericin. They are lipophilic substances. They have the ability to make a complex with cations as potassium "K^+" and facilitate their transport into mitochondria and other biological membranes. They inhibit phosphorylation because they decrease both electrical and pH gradient.

C. **Membrane transport systems:**

The inner mitochondrial membrane is impermeable to most charged or hydrophilic substances. However it contains numerous transport proteins (carrier) that permit passage of specific molecules from the

cytosol to the mitochondrial matrix e.g. ADP - ATP carrier which carriers ADP from cytosol into mitochondria, while carrying ATP from the matrix back to cytosol.

V. *Oxidation of extramitochondrial NADH +H⁺ :*

It is mediated by substrate shuttles (glycerophosphate shuttle and malate-aspartate shuttle).

- The 2 molecules of cytosolic NADH+H⁺ produced by glycolysis cannot penetrate mitochondrial membrane, however, they can be used to produce energy (4 or 6 ATP) by respiratory chain phosphorylation in the mitochondria.
- This can be done by using special carriers for hydrogen of NADH+H⁺. These carriers are either dihydroxyacetone phosphate (glycerophosphate shuttle) or oxaloacetate (aspartate - malate shuttle).

A. Glycerophosphate shuttle:

1) It is important in certain muscles and nerve cells.
2) The final energy produced is 2 x 2 ATP ➔ 4 ATP.
3) **Mechanism** (see the diagram):
   - ➢ The coenzyme of cytosolic glycerol -3- phosphate dehydrogenase is NAD⁺.
   - ➢ The coenzyme of mitochodrial glycerol-3-phosphate dehydrogenase is FAD.
   - ➢ Oxidation of $FADH_2$ in respiratory chain gives 2 ATP.

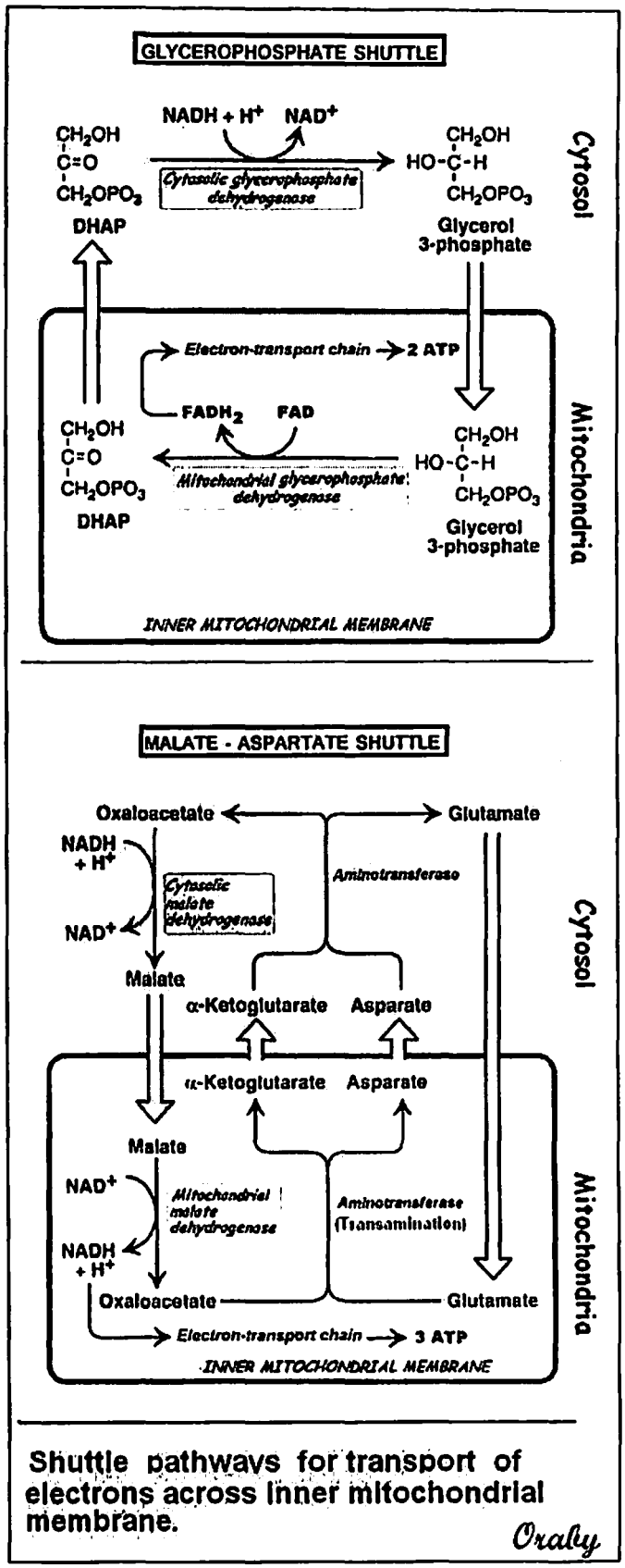
   As glycolysis gives 2 cytosolic NADH + H⁺ → 2 mitochondrial $FADH_2$ → 2 x 2 ATP = 4 ATP.

B. Malate - aspartate shuttle:

1) It is important in other tissues particularly liver and heart.
2) The final energy produced is 2x3 ATP ➔ 6 ATP.
3) **Mechanism:** see diagram
   - ➢ The coenzyme of both cytosolic and mitochondrial malate dehydrogenase is **NAD⁺.**
   - ➢ Oxidation of NADH+H⁺ in respiratory chain gives 3 ATP.

   As glycolysis gives 2 cytosolic NADH+H⁺ → 2 mitochondrial NADH+H⁺ → 2x 3 ATP = 6 ATP.

➢ **Note:** Mitochondrial membrane is impermeable to oxaloacetate. Therefore, oxaloacetate produced inside the mitochondrial cannot be transported to the cytosol again except by a special pathway called: transamination (see protein metabolism).



Shuttle pathways for transport of electrons across inner mitochondrial membrane.

Oraby

VI. *Mitochondrial structure:*

A. **The outer mitochondrial membrane:** is permeable to most small molecules.

B. **The inter-membrane space:** shows no barrier to the substances entering or leaving the mitochondrial matrix.

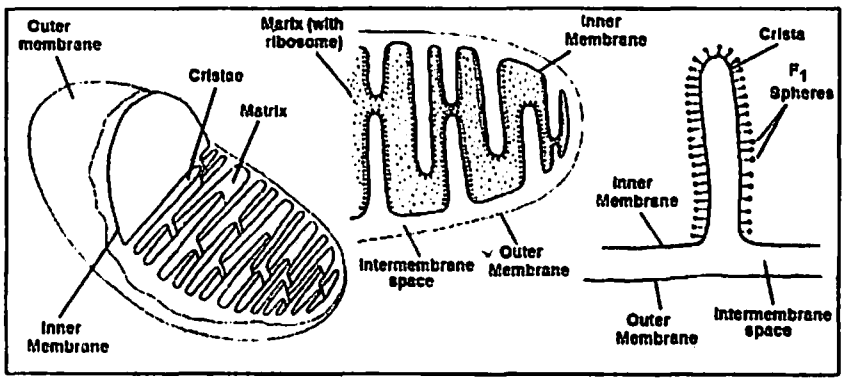
C. **The inner membrane:**

1. The inner mitochondrial membrane is impermeable to most small ions including H^+, Na^+ and K^+, small and large molecules as ATP, ADP, pyruvate and other metabolites important to mitochondrial function. Specialized carriers or transport systems are required to move ions or molecules across this membrane.

2. The inner mitochondrial membrane is highly convoluted. The convolutions are called **cristae** and serve to increase greatly the surface area of the membrane.
3. **ATP synthase complexes:** These complexes of proteins are considered as **inner membrane particles** and are attached to the inner surface of the inner mitochondrial membrane. They include the enzymes of respiratory (electron transport) chain.

D. **Matrix of mitochondrion:** It is a solution like a gel. It is bounded by the inner mitochondrial membrane and contains:

1. The enzymes of tricarboxylic acid cycle (TCA) with exception of succinate dehydrogenase, which is embedded in the inner membrane.
2. The enzymes of B-oxidation of fatty acids.
3. Miscellaneous enzyme systems.



VII) *Mitochondrial functions:*

The following **reactions** occur in the mitochondria:

A. **Carbohydrate metabolism:**

1. Oxidative decarboxylation of pyruvate and α ketoglutarate.
2. Tricarboxylic acid cycle.
3. Part of gluconeogenesis.

B. **Respiratory chain.**

1. Oxidative phosphorylation.
2. Most of ATP formation in the cells (cell battery).

C. **Lipids metabolism:**

1. β-Oxidation of Fatty acids.
2. Mitochondrial synthesis of fatty acids.
3. Ketogenesis.

D. **Protein metabolism:**

1. Transamination.
2. Part of heme synthesis.
3. Part of urea synthesis

Chapter 2 *Carbohydrate Metabolism*

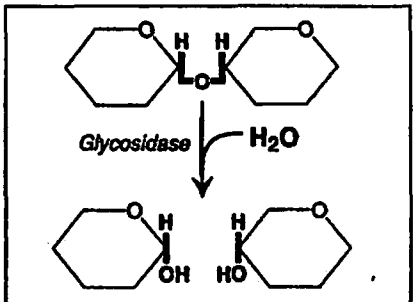
Digestion

I. ***Introduction:*** More than 60% of our foods are carbohydrates.

A. Starch, glycogen, sucrose, lactose and cellulose are the chief carbohydrates in our food.

B. Before intestinal absorption, they are hydrolyzed to monosaccharides (glucose, galactose and fructose).

C. A family of glycosidases that hydrolyzes carbohydrate into their monosaccharide components catalyzes hydrolysis of glycosidic bonds.



Hydrolysis of a glycosidic bond.

II. ***Digestion of carbohydrate by salivary α -amylase (ptylin) in the mouth:***

A. This enzyme is **produced by salivary glands.** Its optimum pH is **6.7.**

B. It is activated by chloride ions (Cl^-).

C. It acts on **cooked starch** and glycogen breaking α 1-4 bonds, converting them into maltose [a disaccharide containing two glucose molecules attached by α 1-4 linkage]. The bond of maltose is not attacked by α- amylase.

D. Because both starch and glycogen also contain α 1-6 bonds, the resulting digest contains **isomaltose** [a disaccharide in which two glucose molecules are attached by α 1-6 linkage].

E. **Because food remains for a short time in the mouth,** digestion of starch and glycogen may be incomplete and gives a partial digestion products called: **starch dextrins** (amylodextrin, erythrodextrin and achrodextrin).

F. Therefore, digestion of starch and glycogen **in the mouth** gives maltose, isomaltose and starch dextrins.

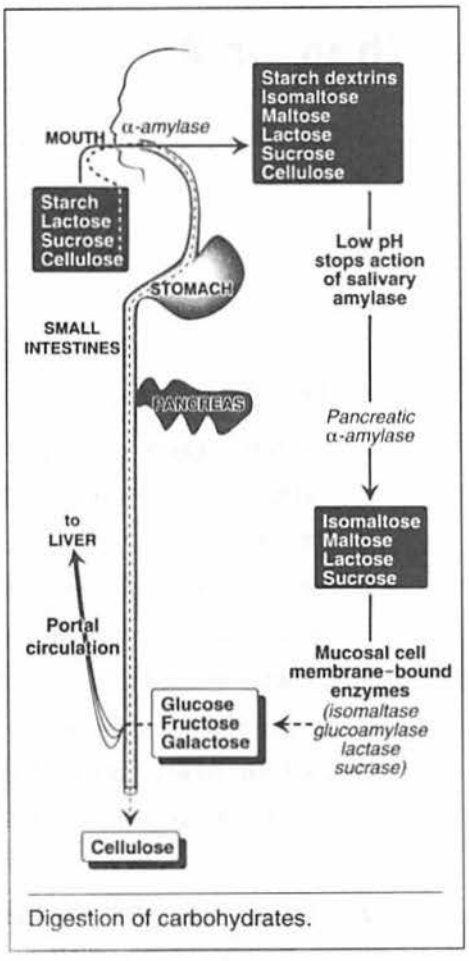
III. ***In the stomach:*** carbohydrate digestion stops temporarily due to the high acidity, which inactivates the salivary α-amylase.

IV. ***Digestion of carbohydrate by pancreatic α- amylase in the small intestine:***

A. α-amylase enzyme is produced by pancreas and acts in small intestine. Its optimum pH is **7.1**.

B. It is also activated by chloride ions.

C. It acts on cooked and uncooked starch, hydrolyzing them into maltose and isomaltose.



Digestion of carbohydrates.

V. ***Final carbohydrate digestion by intestinal enzymes:***

A. The final digestive processes occur at the small intestine and include the action of several disaccharidases. These enzymes are secreted through and remain associated with the brush border of the intestinal mucosal cells.

B. **<u>The disaccharidases include:</u>**

1- **Lactase** (β-galactosidase) which hydrolyses **lactose** into two molecules of glucose and galactose:

$$\text{Lactose} \xrightarrow{\textit{Lactase}} \text{Glucose} + \text{Galactose}$$

2- **Maltase** (α-glucosidase), which hydrolyses **maltose** into two molecules of glucose:

$$\text{Maltose} \xrightarrow{\textit{Maltase}} \text{Glucose} + \text{Glucose}$$

3- **Sucrase** (α-fructofuranosidase), which hydrolyses **sucrose** into two molecules of glucose and fructose:

$$\text{Sucrose} \xrightarrow{\textit{Sucrase}} \text{Glucose} + \text{Fructose}$$

4. **α-dextrinase** which hydrolyzes the (1,6) linkage of **isomaltose.**

Isomaltose —Dextrinase→ Glucose + Glucose

VI. *Digestion of cellulose:*

A. Cellulose contains β(1-4) bonds between glucose molecules.

B. In humans, there is no β (1-4) glucosidase that can digest such bonds. So cellulose passes as such in stool.

C. Cellulose helps water retention during the passage of food along the intestine → producing larger and softer feces → preventing constipation.

Absorption

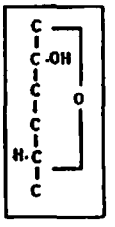
I. *Introduction:*

A. The end products of carbohydrate digestion are **monosaccharides**: glucose, galactose and fructose. They are absorbed from the jejunum to portal veins to the liver, where fructose and galactose are transformed into glucose.

B. Two mechanisms are responsible for absorption of monosaccharides: **active transport** (against concentration gradient i.e. from low to high concentration) and **passive transport (by facilitated diffusion).**

C. For active transport to take place, the structure of sugar should have:

1. **Hexose ring.**
2. **OH group at position 2** at the right side. Both of which are present in glucose and galactose. Fructose, which does not contain -OH group to the right at position 2 is absorbed more slowly than glucose and galactose by passive diffusion (slow process).



3. **A methyl or a substituted methyl group** should be present at carbon 5.

II. *Mechanisms of absorption:*

A. Active transport:

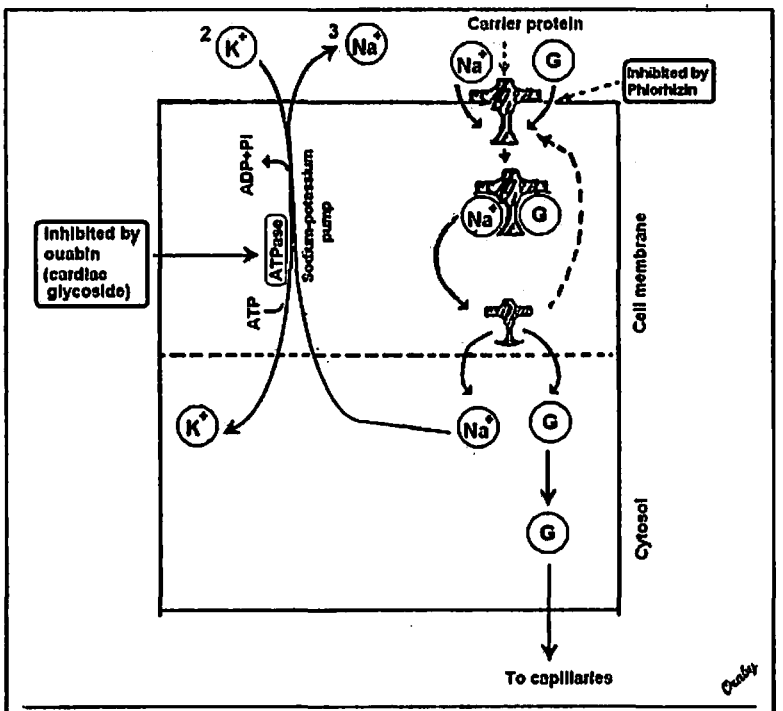
1. Mechanism of active transport:

a) In the cell membrane of the intestinal cells, there is a **mobile carrier protein** called **sodium – dependant glucose**

transporter (SGLT-1). It transports glucose to inside the cell using energy. The energy is derived from sodium-potassium pump. The transporter has 2 separate sites, one for sodium and the other for glucose. It transports them from the intestinal lumen across cell membrane to the cytosol. Then both glucose and sodium are released into the cytosol allowing the carrier to return for more transport of glucose and sodium.

b) The sodium is transported from high to low concentration (with concentration gradient) and at the same time causes the carrier to transport glucose against its concentration gradient. The Na^+ is expelled outside the cell by **sodium – potassium pump,** which needs ATP as a source of energy. The reaction is catalyzed by an enzyme called "**Adenosine triphosphatase** (ATPase)". Active transport is much more faster than passive transport.

c) **Insulin** increases the number of glucose transporters in tissues containing **insulin receptors** e.g. muscles and adipose tissue.



Transport the glucose across the intestinal epithelium. Active glucose transport is coupled to the Na^+ - K^+ pump.

2. **Inhibitors of active transport :**

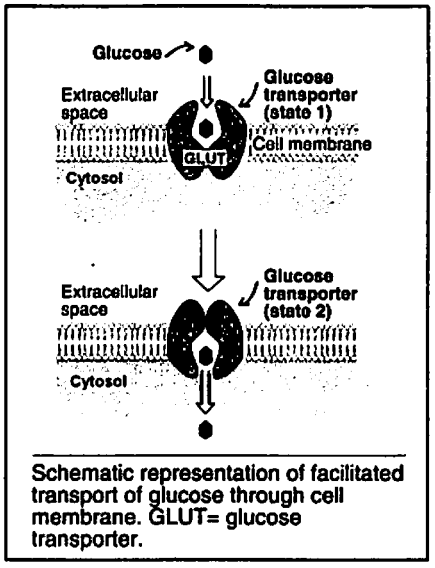
a) **Ouabin (cardiac glycoside):** Inhibits adenosine triphosphatase (ATPase) necessary for hydrolysis of ATP that produces energy of sodium-potassium pump.

b) **Phlorhizin:** Inhibits the binding of sodium in the carrier protein.

B. **Passive transport (facilitated diffusion):**

Sugars pass with concentration gradient i.e. from high to low concentration. It needs **no energy**. It occurs by means of sodium – independent facilitative transporter (GLUT-5). Fructose and pentoses are absorbed by this mechanism. Glucose and galactose can also use the same transporter if the concentration gradient is favorable.

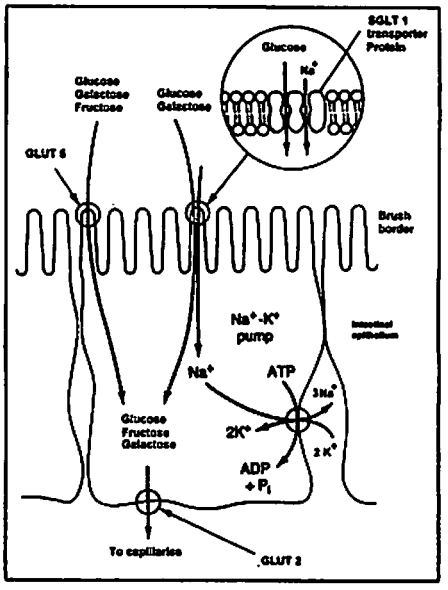
C. There is also **sodium – independent transporter (GLUT-2)**, that facilitates transport of sugars out of the cell i.e. to circulation.



Schematic representation of facilitated transport of glucose through cell membrane. GLUT= glucose transporter.

Summary of types and functions of most important glucose transporters:

	Function	*Site*
SGLT-1	Absorption of glucose by active transport (energy is derived from Na^+-K^+ pump.	Intestine and renal tubules
GLUT-5	Fructose transport · and to a lesser extent glucose and galactose.	Intestine and sperm
GLUT-2	Transport glucose out of intestinal and renal cells → circulation.	-Intestine and renal tubules -β cells of islets-liver



III. *Defects of carbohydrate digestion and absorption:*

A. Lactase deficiency = lactose intolerance:

1. Definition:

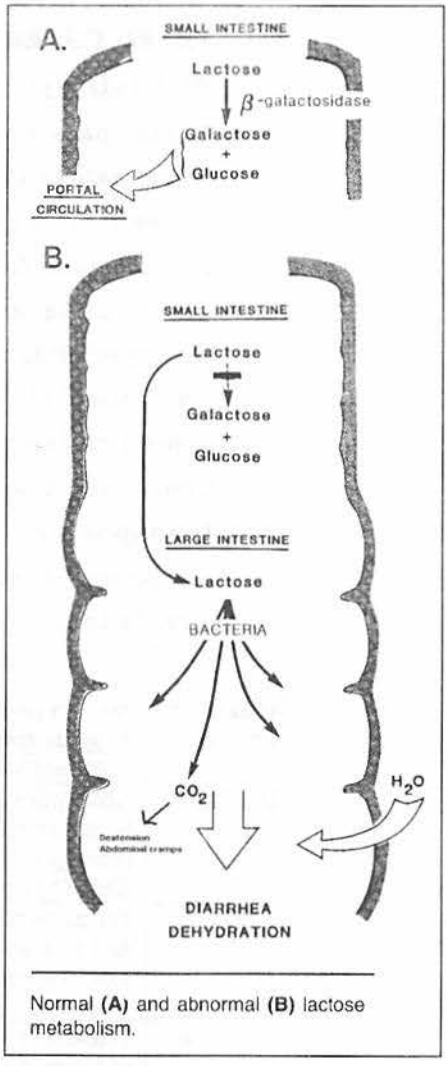
a) This is a deficiency of lactase enzyme which digests lactose into glucose and galactose

b) It may be:

(i) **Congenital:** This occurs very soon after birth (rare).

(ii) **Acquired:** This occurs later on, in life (common).

2. **Effect:** The presence of lactose in intestine causes:
   a) **Increased osmotic pressure:** So water will be drawn from the tissue (causing dehydration) into the large intestine (causing diarrhea).
   b) **Increased fermentation of lactose by bacteria:** Intestinal bacteria ferment lactose with subsequent production of CO_2 gas. This causes distention and abdominal cramps.
3. **Treatment:** Treatment of this disorder is simply by removing lactose (milk) from diet.



Normal **(A)** and abnormal **(B)** lactose metabolism.

B. **Sucrase deficiency:**

It is a rare condition, showing a similar signs and symptoms as lactase deficiency. It occurs early in childhood.

C. **Monosaccharides malabsorption:**

This is a congenital condition in which glucose and galactose are absorbed only slowly due to defect in the GLUT-1. Because fructose is not absorbed by the GLUT-1, its absorption is normal.

IV. *Fate of absorbed sugars:*

Monosaccharides (glucose, galactose and fructose) resulting from carbohydrate digestion are absorbed and undergo the following:

A. **Uptake by liver:**

After absorption the liver takes up sugars, where galactose and fructose are converted into glucose.

B. **Glucose utilization by tissues:** Glucose may undergo one of the following fate:

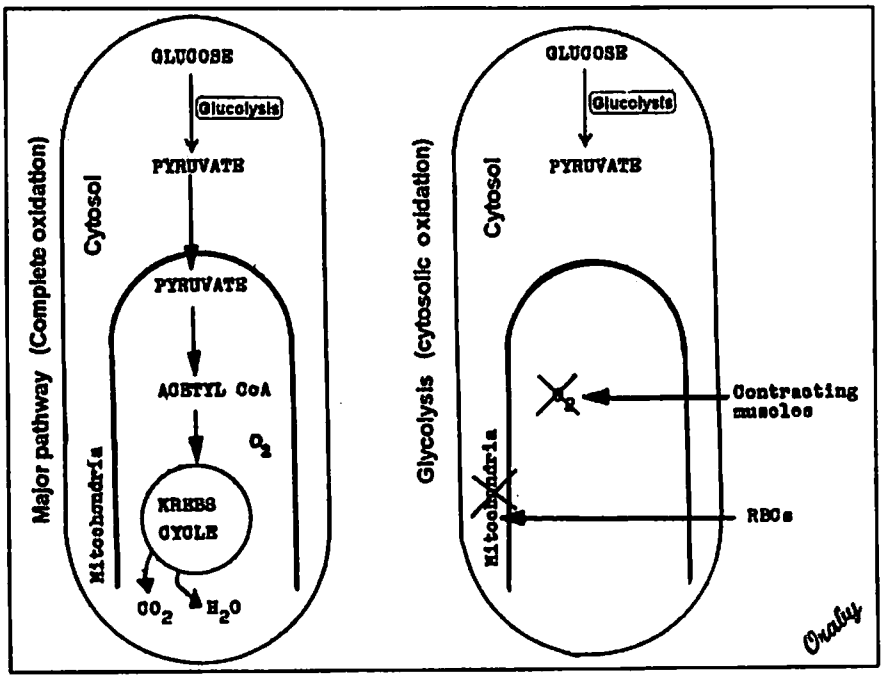
1. **Oxidation:** through
   a) **Major pathways** (glycolysis and Krebs` cycle) mainly for production of energy.
   b) **Pentose phosphate pathway:** for production of pentoses and NADPH + H^+.

c) **Uronic acid pathway:** for production of glucuronic acid. This sugar derivative is used in detoxication and enters in the structure of glycosaminoglycans.

2. **Storage in the form of:**
   a) **Glycogen:** glycogenesis.
   b) **Fat:** lipogenesis.
3. **Conversion:** to substances of biological importance:
   a) Ribose , deoxyribose ➔ RNA and DNA.
   b) Lactose ➔ milk.
   c) Glucosamine and galactosamine ➔ mucopolysaccharides.
   d) Glucuronic acid ➔ glycosaminoglycans and mucopolysaccharides.
   e) Fructose ➔ in semen.

GLUCOSE OXIDATION

Major Pathway



I. *Glycolysis (Embden Meyerhof Pathway):*

A. Definition:

1. Glycolysis means oxidation of glucose to give pyruvate (in the presence of oxygen) or lactate (in the absence of oxygen).

B. Site:

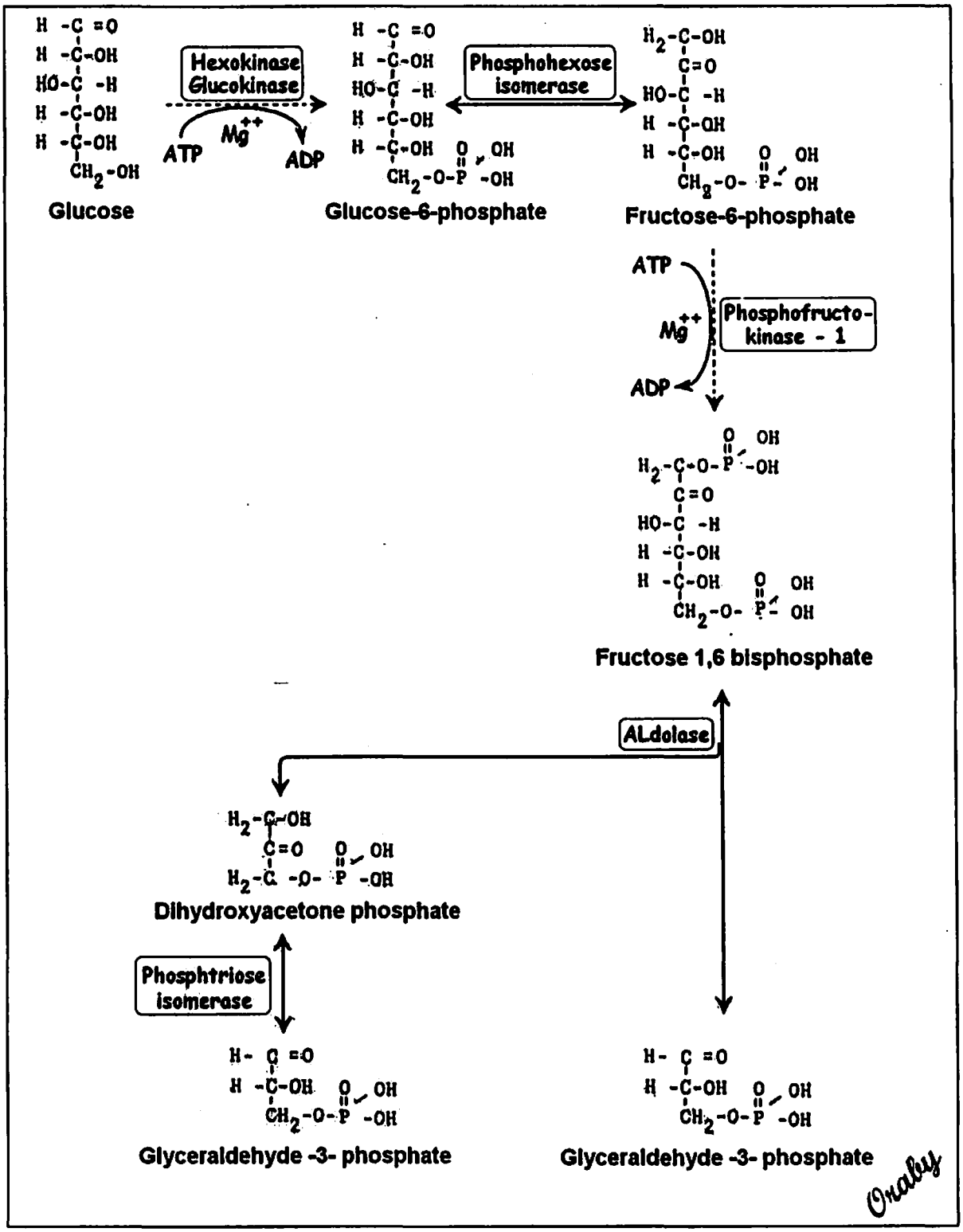
1. **Intracellular location:** cytosol.
2. **Organ location:** all tissue cells, but it is of physiological importance in:

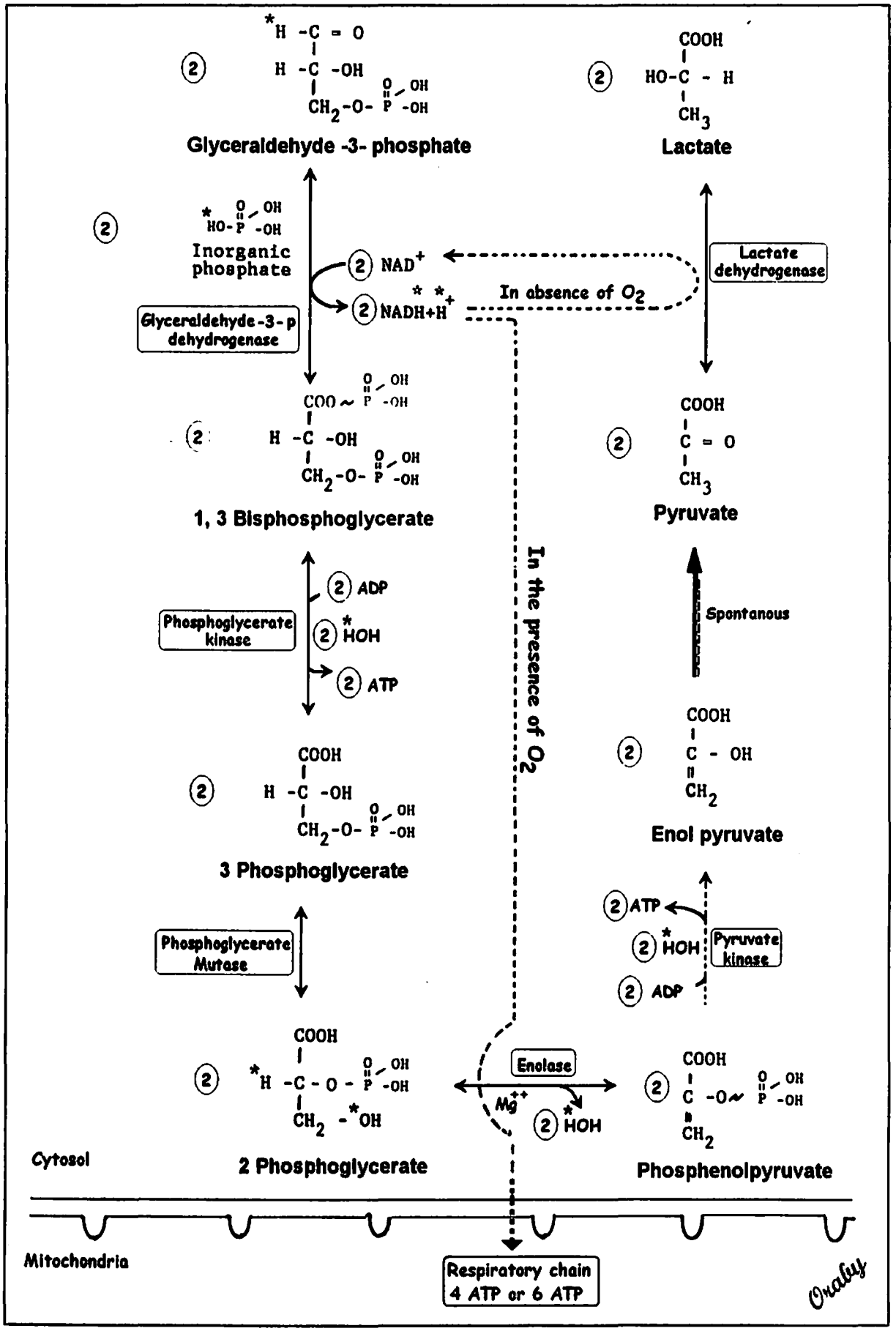
a) **Tissues with no mitochondria:** e.g. mature RBCs.

b) **Tissues with few mitochondria:** e.g. Testes and leucocytes.

c) **Tissues undergo frequent oxygen lack:** skeletal muscles especially during exercise.

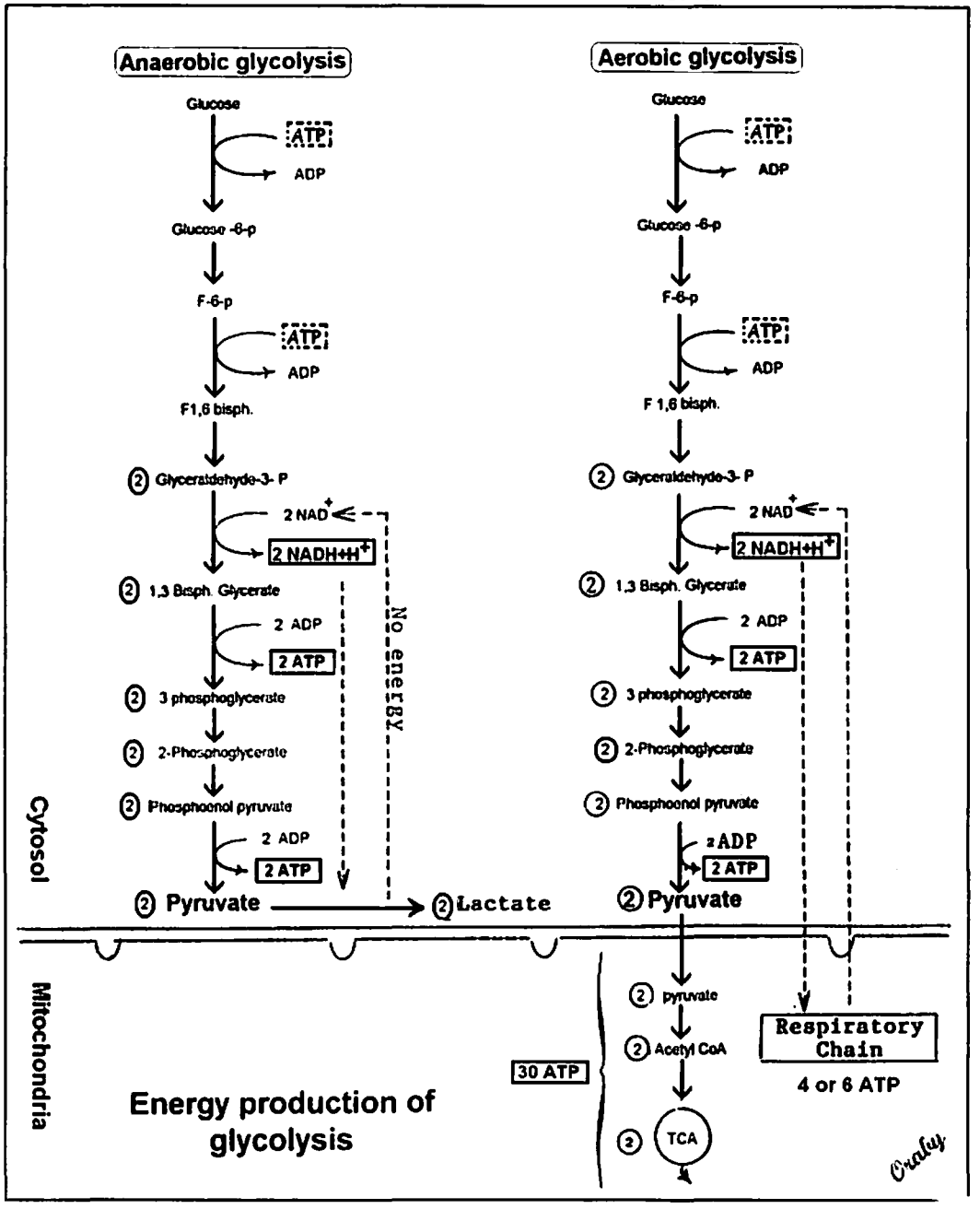
C. **Steps:**




Glyceraldehyde -3- phosphate
Lactate
Inorganic phosphate
2 NAD+
2 NADH+H+
In absence of O2
Lactate dehydrogenase
Glyceraldehyde-3-p dehydrogenase
1, 3 Bisphosphoglycerate
Pyruvate
2 ADP
2 HOH
2 ATP
Phosphoglycerate kinase
In the presence of O2
Spontanous
3 Phosphoglycerate
Enol pyruvate
Phosphoglycerate Mutase
2 ATP
2 HOH
2 ADP
Pyruvate kinase
Enolase
Mg++
2 HOH
2 Phosphoglycerate
Phosphenolpyruvate
Cytosol
Mitochondria
Respiratory chain
4 ATP or 6 ATP

Stages of glycolysis:

1. **Stage one (the energy requiring stage):**
   a) One molecule of **glucose** is converted into two molecules of **glyceraldehyde-3-phosphate.**
   b) These steps requires **2 molecules of ATP** (energy loss)
2. **Stage two (the energy producing stage):**
   a) The 2 molecules of **glyceraldehyde-3-phosphate** are converted into 2 **pyruvate** molecules (aerobic glycolysis) or 2 **lactate** molecules (anaerobic glycolysis).
   b) These steps produce ATP molecules (energy production).



D. Energy (ATP) production of glycolysis:

ATP production = ATP produced - ATP utilized

	ATP produced	ATP utilized	Net energy
In absence of oxygen (anaerobic glycolysis)	**4 ATP** (Substrate level phosphorylation) • 2ATP from 1,3 BPG. • 2ATP from phosphoenol pyruvate	**2 ATP** • From glucose to glucose-6-p. • From fructose-6-p to fructose 1,6 bisp.	**2 ATP**
In presence of oxygen (aerobic glycolysis)	**4 ATP** (Substrate level phosphorylation) • 2ATP from 1,3 BPG. • 2ATP from phosphoenol pyruvate + **4 ATP or 6 ATP** *(From oxidation of 2 NADH + H in mitochondria).	**2 ATP** • From glucose to glucose-6-p. • From fructose-6-p to fructose 1,6 bisphosphate.	**6ATP or 8ATP**

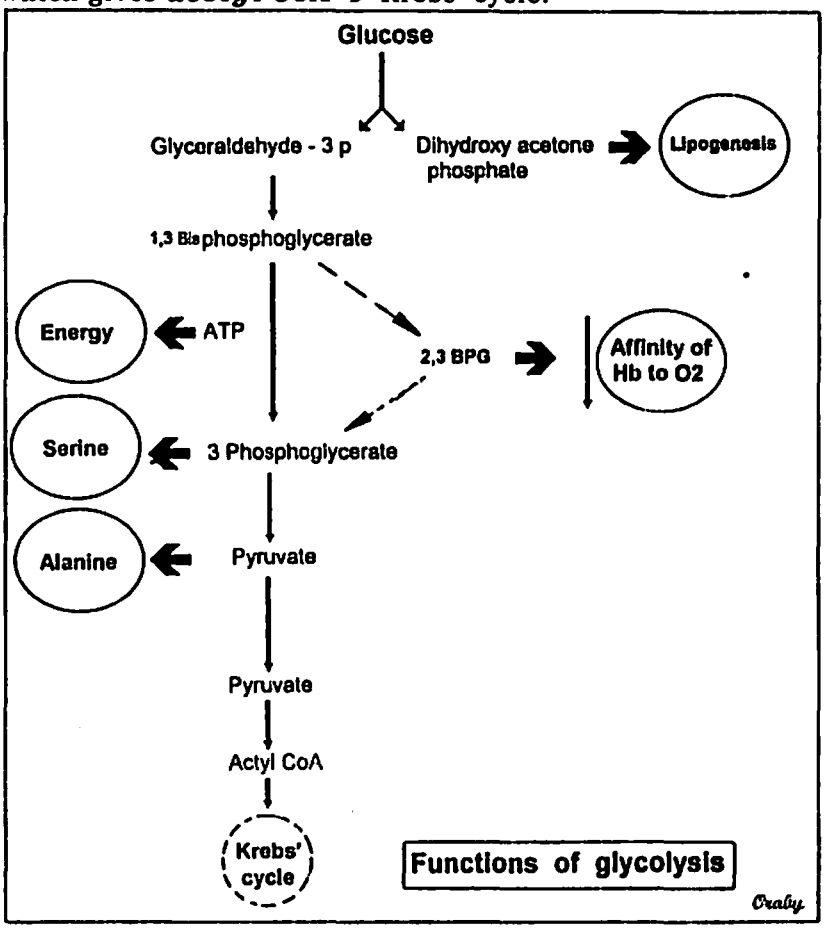
E. Oxidation of extramitochondrial NADH+H$^+$:

1. The 2 molecules of cytosolic NADH+H$^+$ cannot penetrate mitochondrial membrane, however, they can be used to produce energy (4 or 6 ATP) by respiratory chain phosphorylation in the mitochondria (see chapter I).

F. Biological importance (functions) of glycolysis:

1. **Energy production :**
   a) Anaerobic glycolysis gives 2 ATP.
   b) Acrobic glycolysis gives 8 ATP.
2. **Oxygenation of tissues:**
   Through formation of 2,3 bisphosphoglycerate, which decreases the affinity of Hemoglobin to O_2.
3. **Provides important intermediates:**
   a) Dihydroxyacetone phosphate: may give glycerol-3-phosphate, which is used for ***synthesis of triacylglycerols and phospholipids*** (lipogenesis).
   b) 3 Phosphoglycerate: which may be used for ***synthesis of amino acid serine.***
   c) Pyruvate: which may be used in ***synthesis of amino acid alanine.***

4. Aerobic glycolysis provides the mitochondria with ***pyruvate***, which gives ***acetyl CoA*** → Krebs' cycle.

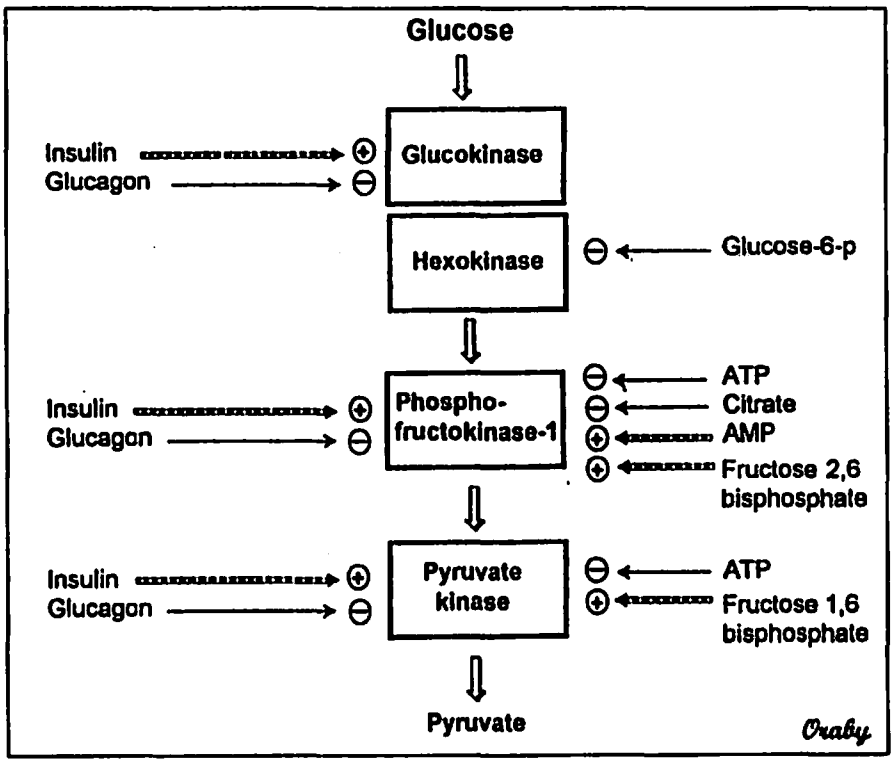


G. Regulation of glycolysis:

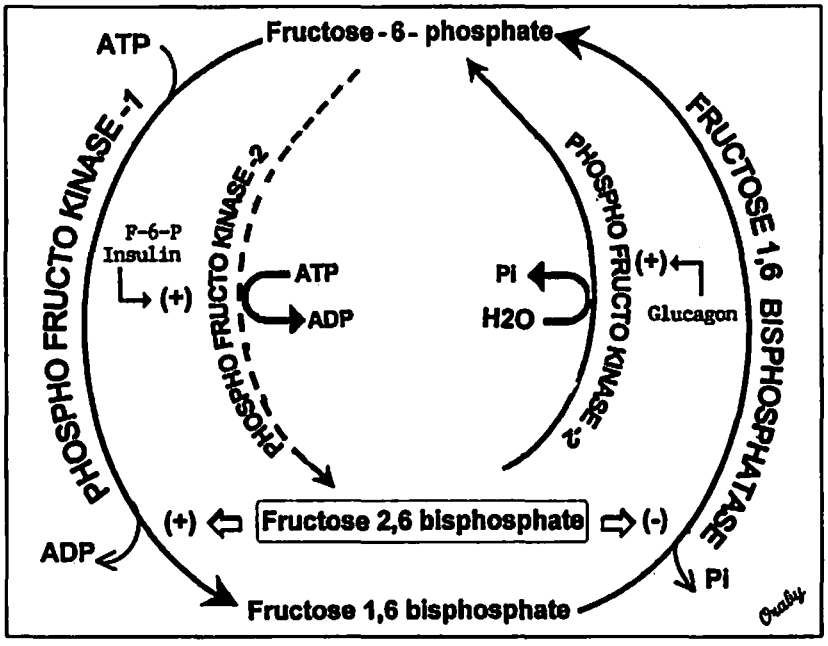
The rate of glycolysis is regulated by controlling of the three irreversible enzymes (key enzymes). These enzymes catalyze what is called committed reactions of the pathway. These enzymes are; glucokinase (hexokinase), phosphofructokinase-1 and pyruvate kinase.

1. **Hormonal regulation:**
   a) **Insulin:** ***Stimulates*** synthesis of all key enzymes of glycolysis. It is secreted after meal (in response to high blood glucose level).
   b) **Glucagon:** ***Inhibits*** the activity of all key enzymes of glycolysis. It is secreted in response to low blood glucose level.
2. **Energy regulation:**
   a) **High level of ATP** inhibits PFK-1 and pyruvate kinase.
   b) **High level of ADP and AMP** stimulate PFK-1.

3. **Substrate regulation:**
   a) Glucose-6-phosphate inhibits hexokinase (and not glucokinase).
   b) Fructose 2,6 bisphosphate stimulates phosphofructokinase-1.
   c) Citrate inhibits phosphofructokinase-1.
   d) Fructose 1,6 bisphosphate stimulates pyruvate kinase.
4. **Fructose 2,6 bisphosphate:**
   a) This substrate is produced from fructose-6-phosphate by reaction catalyzed by: phosphofructokinase-2 (PFK-2) enzyme.
   b) Fructose 2,6 bisphosphate stimulates glycolysis by allosteric stimulation of phosphofructokinase-1. It also inhibits gluconeogenesis by inhibiting fructose 1,6 bisphosphatase enzyme.



   c) PFK-2 (present mainly in liver) has bi-functional role:
      1) **Conversion of fructose-6-phosphate into fructose 2,6 bisphosphate.** This reaction is stimulated **after meal** by fructose-6-phosphate and insulin.
      2) **Conversion of fructose 2,6 bisphosphate into fructose-6-phosphate** (exactly as phosphatase). This reaction is stimulated **during fasting** by glucagon hormone.



H. Differences between aerobic and anaerobic glycolysis:

	Aerobic	Anaerobic
1-End product	Pyruvate	Lactate
2-Energy	6 or 8 ATP	2 ATP
3-Regeneration of NAD^+	Through respiratory chain in mitochondria	Through lactate formation
4-Availability to TCA in mitochondria	Available and 2 pyruvate can be oxidized to give 30 ATP	Not available as lactate is a cytosolic substrate

I. Substrate level phosphorylation in glycolysis:

This means phosphorylation of ADP to ATP at the reaction itself.

In glycolysis there are 2 examples:

- 1,3 Bisphosphoglycerate + ADP → 3 Phosphoglycerate + ATP
- Phospho-enolpyruvate + ADP → Enolpyruvate + ATP

J. Importance of lactate production in anaerobic glycolysis:

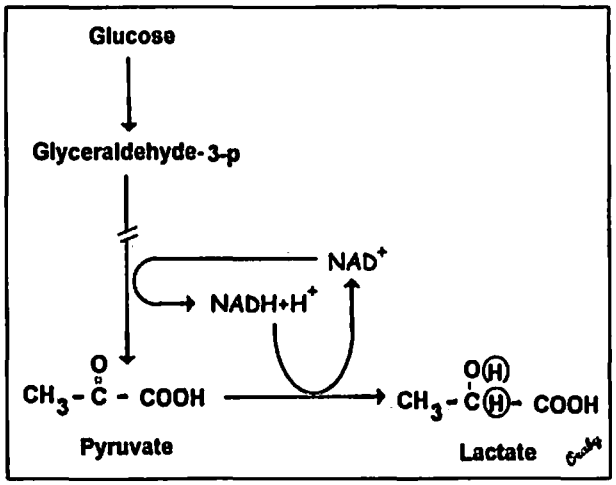
1. In absence of oxygen, lactate is the end product of glycolysis:

   Glucose → Pyruvate → Lactate

2. In absence of oxygen, $NADH+H^+$ is not oxidized by the respiratory chain, thus:

   The conversion of pyruvate to lactate is the mechanism for regeneration of NAD^+.

3. This helps continuity of glycolysis, as the generated NAD^+ will be used once more for oxidation of another glucose molecule.



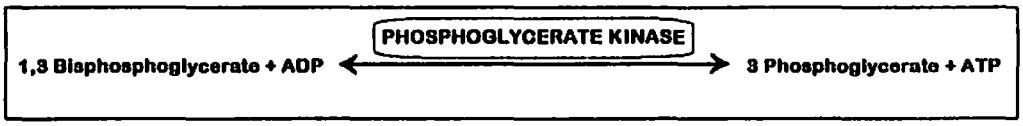
K. Special features of glycolysis in RBCs:

1. Mature RBCs contain **no mitochondria**, thus:
   a) They depend *only* upon **glycolysis** for energy production (=2 ATP).
   b) **Lactate** is always the end product.
2. Glucose uptake by RBCs is **independent on insulin** hormone.
3. **Reduction of met-hemoglobin:** Met-hemoglobin binds oxygen irreversibly. Glycolysis produces $NADH+H^+$, which used for reduction of met-hemoglobin in red cells, into hemoglobin. This reaction is catalyzed by **cytochrome b_5-met-haemoglobin reductase** system (cyt b_5):

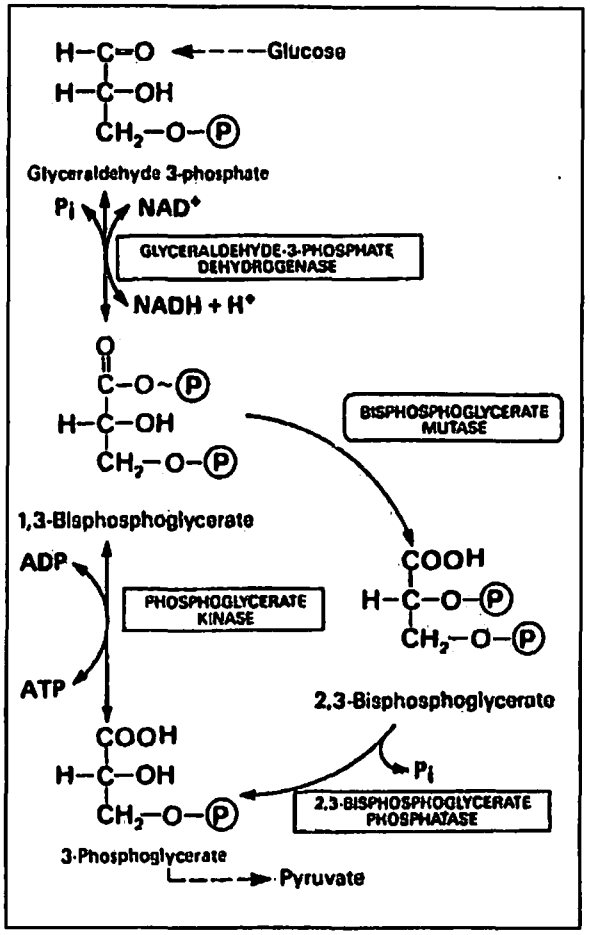
Met-Hemoglobin (Fe^{+++}) + $NADH+H^+$ → Hemoglobin (Fe^{++}) + NAD^+

4. **2,3 Bisphosphoglycerate:**
   a) The RBCs have the ability to form 2,3 bisphosphoglycerate (2,3 BPG) through what is called: Rapoport-Luebering cycle or 2,3 bisphosphoglycerate cycle. **2,3 BPG ↓ affinity of hemoglobin to O_2** → Good oxygenation of tissues.
   b) **Mechanism:**

In the erythrocytes of many mammalian species the reaction

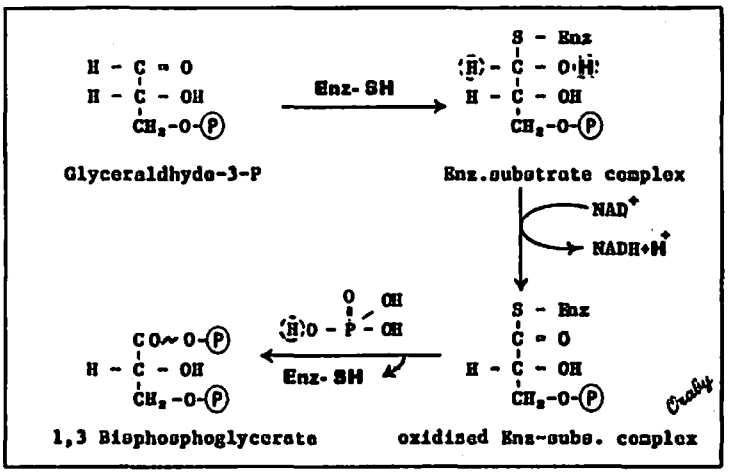


Is bypassed by other 2 reactions without producing energy (ATP) as follows:



L. Mechanism of oxidation of glyceraldhyde-3-phosphate:

1. Glyceraldhyde-3-phosphate is oxidized by glyceraldhyde-3-p dehydrogenase enzyme.
2. This enzyme contains -SH group in its active center:



M. Reversibility of glycolysis: Reversible reaction means that the same enzyme can catalyze the reaction in both directions.

1. All reactions of glycolysis –except 3- are reversible.

2. The 3 irreversible reactions (those are catalyzed by kinase enzymes) are:
   a) Glucose-6-phosphate → Glucose
   b) F 1,6 Bisphosphate → Fructose-6-phosphate
   c) Pyruvate → Phosphoenol pyruvate.
3. During fasting, glycolysis is reversed for synthesis of glucose from non-carbohydrate sources as lactate. This mechanism is called: ***gluconeogenesis.***

N. **Comparison between glucokinase and hexokinase enzymes:**

	Glucokinase	Hexokinase
1.Site	Liver only	All tissue cells
2.Affinity to glucose	Low affinity(high km) i.e. it acts only in the presence of high blood glucose concentration.	High affinity (low km) i.e. it acts even in the presence of low blood glucose concentration.
3.Substrate	Glucose only	Glucose, galactose and fructose
4.Effect of insulin	Induces synthesis of glucokinase	No effect
5.Effect of glucose-6-phosphate	No effect	Allosterically inhibits hexokinase
6.Function	Acts in liver after meals. It removes glucose coming in portal circulation, converting it into glucose-6-phosphate.	It phosphorylates glucose inside the body cells . This makes glucose concentration more in blood than inside the cells . This leads to continuous supply of glucose for the tissues even in the presence of low blood glucose concentration.

O. **<u>Lactate dehydrogenase</u>:**

1. It is an enzyme which catalyzes the reaction:

   Lactate ⇆ Pyruvate

2. ***This reaction helps the re-oxidation of NADH, H^+ into NAD^+.***
3. It has 5 isoenzymes: LD_1, LD_2, LD_3, LD_4 and LD_5.
4. **Medical importance :**

   Estimation of the activity of lactate dehydrogenase enzyme in plasma helps the diagnosis of heart and liver diseases:

   a) **LD_1:** Elevated in some heart diseases e.g. **myocardial infraction.**
   b) **LD_5:** Elevated in some liver diseases as **acute viral hepatitis.**

P. **In vitro inhibition of glycolysis:**

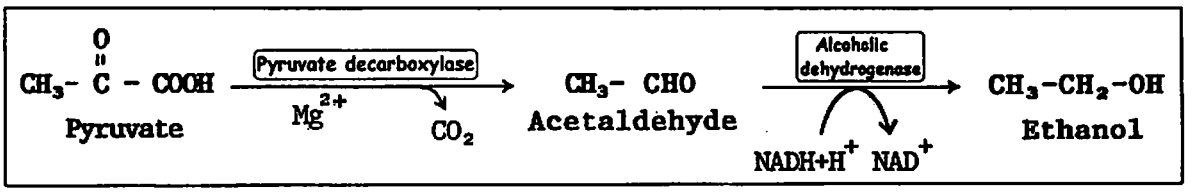
1. **Arsenate:** by competing with inorganic phosphate in the reaction:

   Glyceraldhyde-3-p → 1,3 bisphosphoglycerate

2. **Iodoacetate:** by inhibiting glyceraldhyde-3-p dehydrogenase.
3. **Fluoride:** Inhibits **enolase** enzyme. Clinical laboratories use fluoride to inhibit glycolysis by adding it to the blood before measuring blood glucose.

Q. **Fermentation:**

1. **Definition:** This is conversion of glucose into ethanol by yeast enzymes.
2. Pyruvate is formed by the same series of reactions of glycolysis. Then pyruvate is converted into acetaldehyde, then ethanol as follows:
3. Thus the end product of fermentation is CO_2 and ethanol.



R. **Sources and fate of lactate:**

1. **Sources:**

   From glycolysis especially in RBCs due to absence of mitochondria and muscle during exercises due to oxygen lack.

2. **Fate:**

   a) **Glucose formation:** [through lactic acid cycle (**Cori cycle**)]:

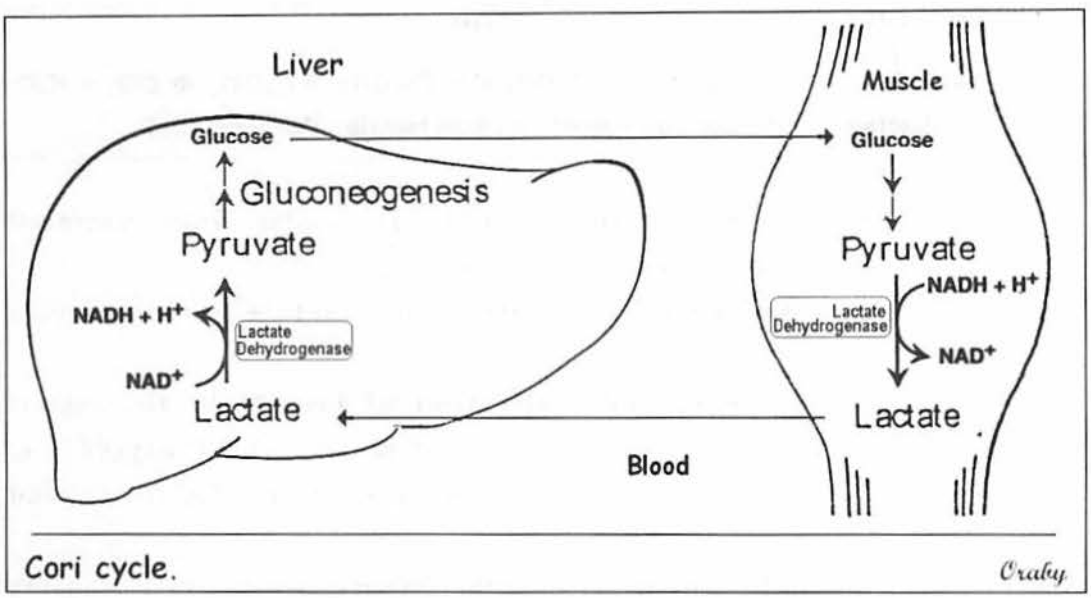
   1) Lactate formed in muscles and RBCs may diffuse to the blood then to the liver.
   2) In the liver, lactate is converted to glucose by gluconeogenesis. Glucose may diffuse back to the blood, then to red cells or muscles to be used for production of energy. This cycle is called: **Lactic acid cycle or Cori cycle.**

Definition of Cori cycle: It is the conversion of glucose into lactate in peripheral tissues, followed by conversion of lactate into glucose in liver.

b) **Conversion into pyruvate:** If oxygen gets available, lactate is converted into pyruvate, which proceeds into Krebs cycle.

c) Lactate may be accumulated in muscles causing **muscle fatigue.**

d) Lactate is **excreted** in urine and sweat.



Cori cycle.

S. **Clinical aspects of glycolysis:**

There are many diseases associated with impaired glycolysis. They include:

1. ***Pyruvate kinase deficiency.***
2. ***Hexokinase deficiency.***
3. ***Lactic acidosis.***

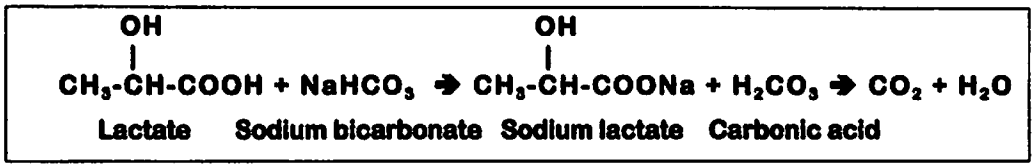
1. Pyruvate kinase (PK) deficiency:

a) This leads to excessive hemolysis of RBCs ➔ leading to hemolytic anemia.

b) Genetic deficiency of PK enzyme causes decrease in the rate of glycolysis and decreased production of ATP.

c) ATP is required for Na^+ -K^+ ATPase, which is important for stability of RBCs.

2. **Hexokinase deficiency:**
   It leads to hemolytic anemia due to decrease ATP production. The mechanism is similar to that of PK deficiency.
3. **Lactic acidosis:**
   a) **Definition and mechanism of lactic acidosis :**
      1) It is the lowered blood pH and bicarbonate levels due to increased blood lactate above normal level.
      2) This depletes bicarbonate ➔ ↓ pH of blood ➔ Lactic acidosis ➔ may lead to coma.



   b) **Causes of lactic acidosis:** It results from increased formation or decreased utilization of lactate.
      1) **Increased formation of lactate** as in severe muscular exercises.
         ➢ **Decreased utilization of lactate in tissues:** it occurs in cases of anoxia or lack of oxygen e.g. myocardial infarction, respiratory disorders, and anemia.
      2) **Phenformin:** is oral hypoglycemic drug, causing excessive anaerobic oxidation of glucose and excess lactate production.

II. *Mitochondrial pathway for glucose oxidation:*

Complete oxidation of glucose occurs in both cytosol (glycolysis) and mitochondria (Krebs` cycle). In the presence of O_2, pyruvate (the end product of glycolysis) passes by special pyruvate transporter into mitochondria which proceeds as follows:

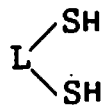
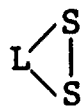
- Oxidative decarboxylation of pyruvate to acetyl CoA.
- Acetyl CoA is then oxidized completely to CO_2, H_2O through Krebs` cycle.

A. Oxidative decarboxylation of pyruvate to acetyl coenzyme A (= acetyl CoA):

1. **Enzyme:** ***Pyruvate dehydrogenase (PDH) complex:***
   a) This enzyme complex contains 3 subunits, which catalyzes the reaction in 4 steps. **These subunits are:** pyruvate decarboxylase, dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase.

b) This enzyme needs 5 coenzymes (all are vitamin B complex derivatives):

1) Vitamin B_1 = Thiamin pyrophosphate = TPP.

2) Lipoic acid = (reduced)  . (oxidized) 

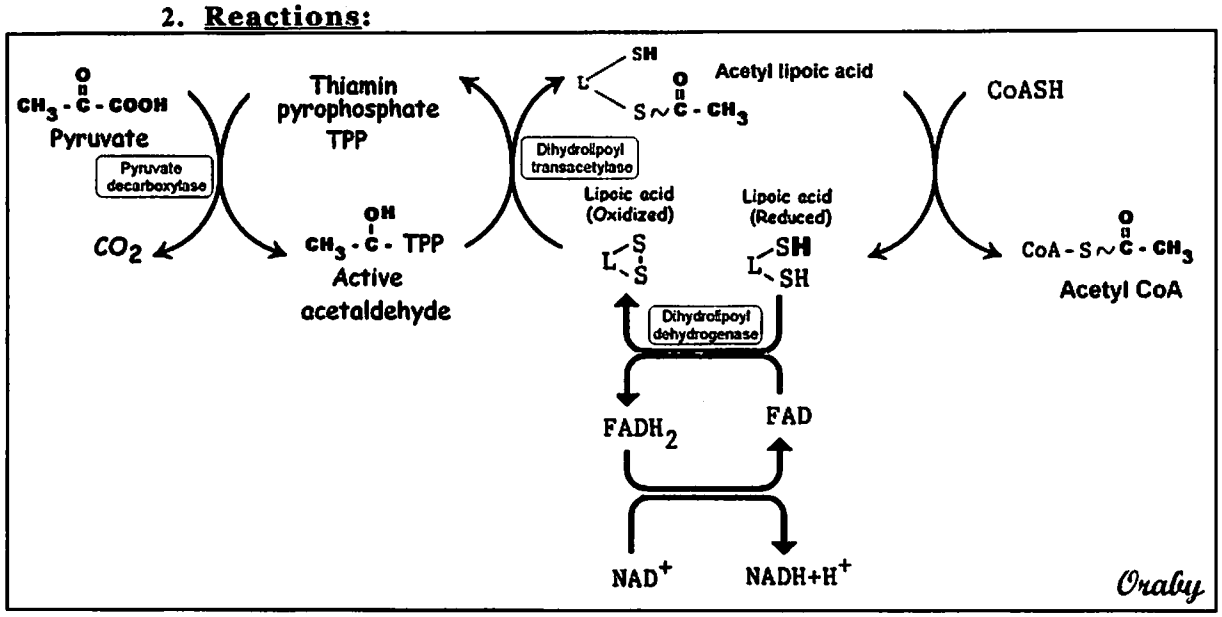
3) Coenzyme A = CoASH.

4) Flavin adenine dinucleotide = FAD.

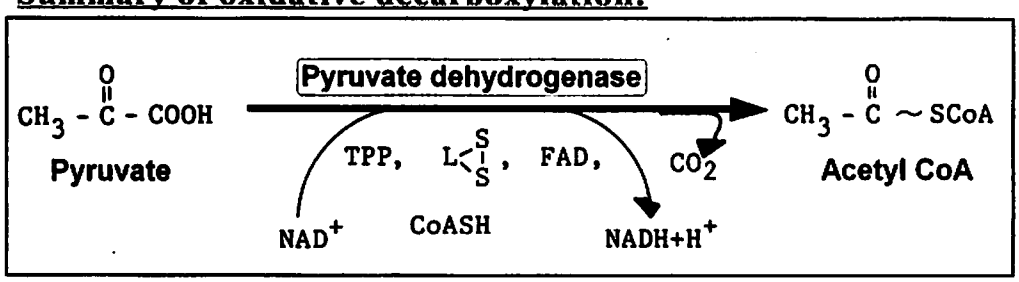
5) Nicotinamide adenine dinucleotide = NAD^+.

c) **Location: PDH** is located within the mitochondrial matrix.

2. **Reactions:**



Summary of oxidative decarboxylation:



3. **Energy production:** ➔ **(3 ATP)**

Oxidative decarboxylation of pyruvate to acetyl CoA produces one molecule of $NADH,H^+$. This produces 3 ATP molecules through respiratory chain phosphorylation.

4. **Regulation of oxidative decarboxylation (PDH):**

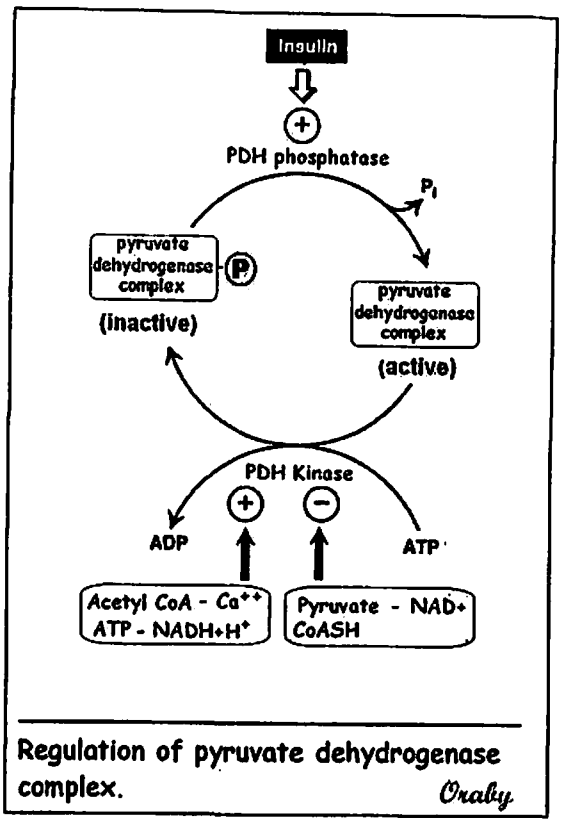
a) PDH exists in two forms: Phosphorylated (inactive) and dephosphorylated (active).

b) Pyruvate dehydrogenase (PDH) kinase enzyme converts active into inactive PDH enzyme.

c) PDH Phosphatase enzyme converts inactive into active PDH enzyme.

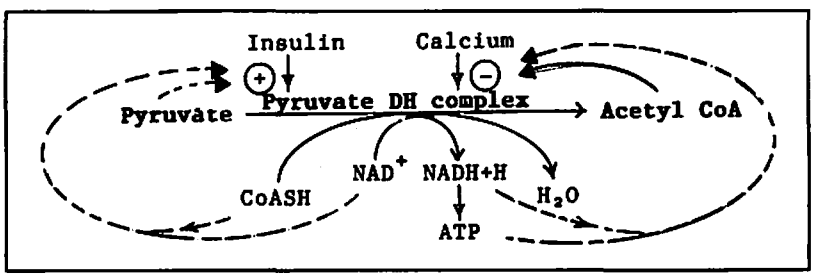
1) **Factors stimulating [+] PDH:**
   - ➢ Pyruvate.
   - ➢ CoASH.
   - ➢ NAD^+.
   - ➢ Insulin hormone.

2) **Factors inhibiting [-] PDH:**
   - ➢ $NADH,H^+$.
   - ➢ ATP.
   - ➢ Acetyl CoA.
   - ➢ Calcium ions.



Regulation of pyruvate dehydrogenase complex. Oraby

Summary of regulation:

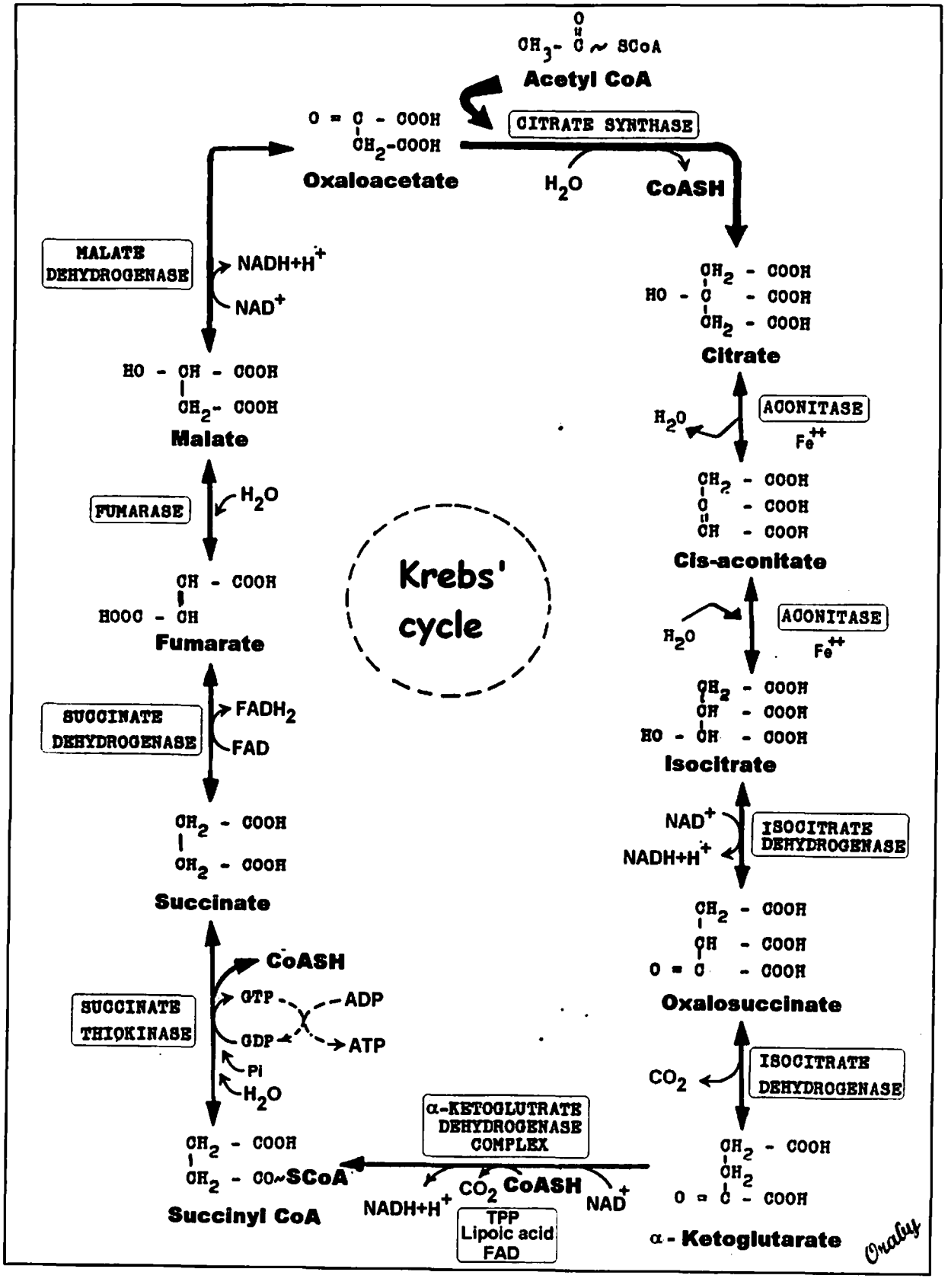


5. **In vitro inhibition of PDH:**
   a) Arsenic.
   b) Thiamin (B_1) deficiency.

B. **Krebs` cycle** [also known as citric acid cycle **(CAC)** or tricarboxylic acid cycle **(TCA) or** catabolism of acetyl CoA]:

1. **Definition:** TCA is a series of reactions in which acetyl CoA is oxidized into CO_2, H_2O and energy.
2. **Location:** Mitochondria.

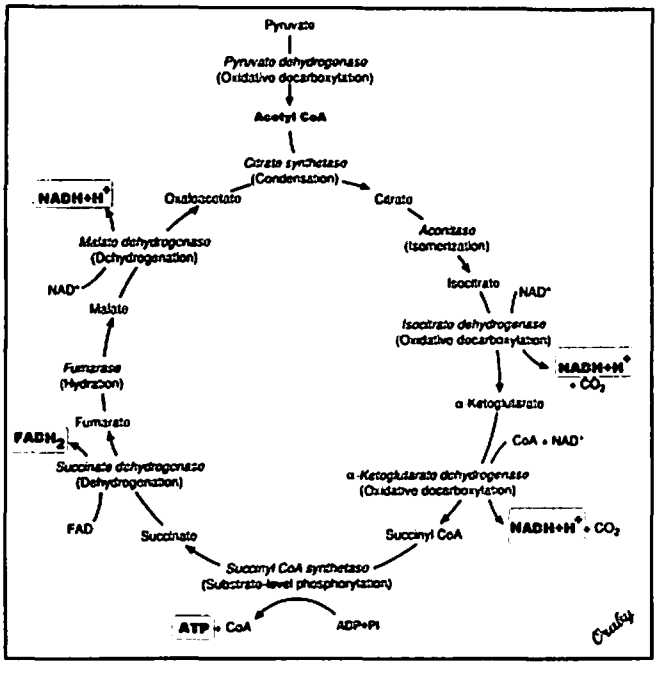
3. **Steps:**


Acetyl CoA
CITRATE SYNTHASE
Oxaloacetate
CoASH
MALATE DEHYDROGENASE
NADH+H+
NAD+
Malate
Citrate
FUMARASE
ACONITASE
Fe++
Cis-aconitate
Krebs' cycle
Fumarate
Isocitrate
SUCCINATE DEHYDROGENASE
FADH2
FAD
ISOCITRATE DEHYDROGENASE
Succinate
Oxalosuccinate
CoASH
SUCCINATE THIOKINASE
GTP
GDP
ADP
ATP
Pi
ISOCITRATE DEHYDROGENASE
α-KETOGLUTRATE DEHYDROGENASE COMPLEX
Succinyl CoA
TPP
Lipoic acid
FAD
α - Ketoglutarate

3. **Steps:**
   a) The enzymes of TCA cycle are present in the mitochondrial matrix either free or attached to the inner surface of the mitochondrial membrane.
   b) The cycle is started by acetyl CoA (2 carbons) and oxaloacetate (4 carbons) to form citrate (6 carbons). It ends by oxaloacetate (4 carbons).

> **The difference between the starting compound (citrate, 6 carbons) and the ending compound (oxaloacetate, 4 carbons) is 2 carbons that are removed in the form of 2 CO_2. These 2 carbons are derived from acetyl CoA. For this reason acetyl CoA is completely catabolized in TCA and never give glucose.**

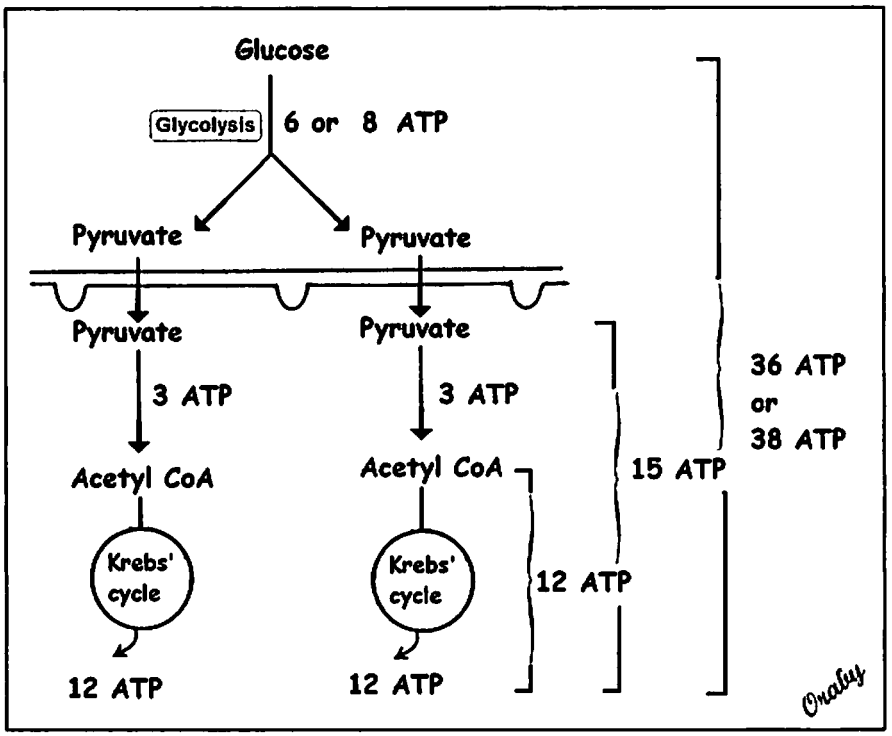
4. **Energy production of TCA:** (Energy produced by catabolism of acetyl CoA):
   a) Oxidation of one molecule of acetyl CoA in TCA produces 12 ATP molecules, 11 by respiratory chain phosphorylation and 1 by substrate level phosphorylation as follows :



Enzyme	Method of ATP production	No. of ATP
Isocitrate dehydrogenase	Oxidation of NADH+H+ by respiratory chain phosphorylation	**3 ATP**
α-Ketoglutarate dehydrogenase	Oxidation of NADH+H+ by respiratory chain phosphorylation	**3 ATP**
Succinyl CoA thiokinase	Substrate level phosphorylation	**1 ATP**
Succinate dehydrogenase	Oxidation of FADH by respiratory chain phosphorylation	**2 ATP**
Malate dehydrogenase	Oxidation of NADH+H+ by respiratory chain phosphorylation	**3 ATP**
Total =		**12 ATP**

b) Energy production of complete oxidation of one molecule of glucose:

- Glucose oxidation ➔ 36 or 38 ATP.
- Pyruvate oxidation ➔ 15 ATP.
- Acetyl CoA ➔ 12 ATP



5. Oxidative decarboxylation of α-ketoglutarate to succinyl CoA:

It is similar to the conversion of pyruvate to acetyl CoA.

a) **Enzymes:** α-ketoglutarate dehydrogenase complex.

b) **Coenzymes:** TPP, Lipoic acid, CoASH, FAD and NAD^+.

6. Functions (significance) of TCA:

TCA cycle is **amphibolic** i.e. it has catabolic (breakdown) and anabolic (formation) functions.

Energy: 12 ATP

Catabolic functions: Oxidation of carbohydrate, lipids and proteins.

Anabolic functions: Formation of:

* Amino acids
* Glucose
* Heme
* Fatty acid and cholesterol
* CO_2

a) **Production of energy** (12 ATP).

b) **Catabolic functions:** TCA is the final common pathway for oxidation of carbohydrates, fats and proteins (amino acids).

c) **Anabolic functions:** Formation of:

1) **Amino acids :**

α-Ketoglutarate —*Transamination*→ Glutamate.

Oxaloacetate —*Transamination*→ Aspratate.

2) **Glucose:** e.g.

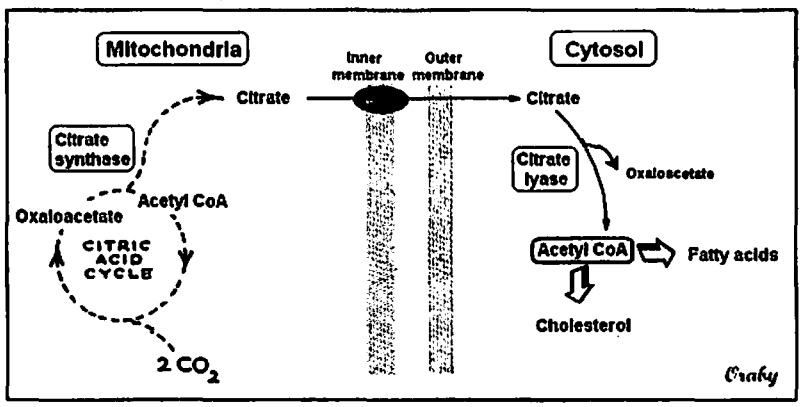
α-ketoglutarate —*Gluconeogenesis*→ Glucose.

3) **Heme synthesis :**

Succinyl CoA ➔ Heme.

4) **Fatty acid and cholesterol: from acetyl CoA (synthesized in cytosol):** (see the figure).

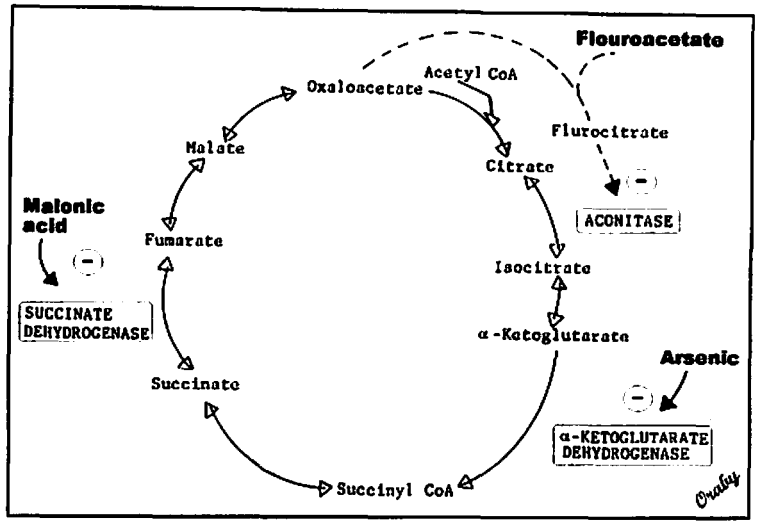
(Note that acetyl CoA is produced in mitochondria. The inner mitochondrial membrane is impermeable to acetyl CoA. To overcome this impermeability, acetyl CoA combines with oxaloacetate to form citrate. Citrate (diffuses to cytosol) ➔ Oxaloacetate + Acetyl CoA ➔ Fatty acid and cholesterol.



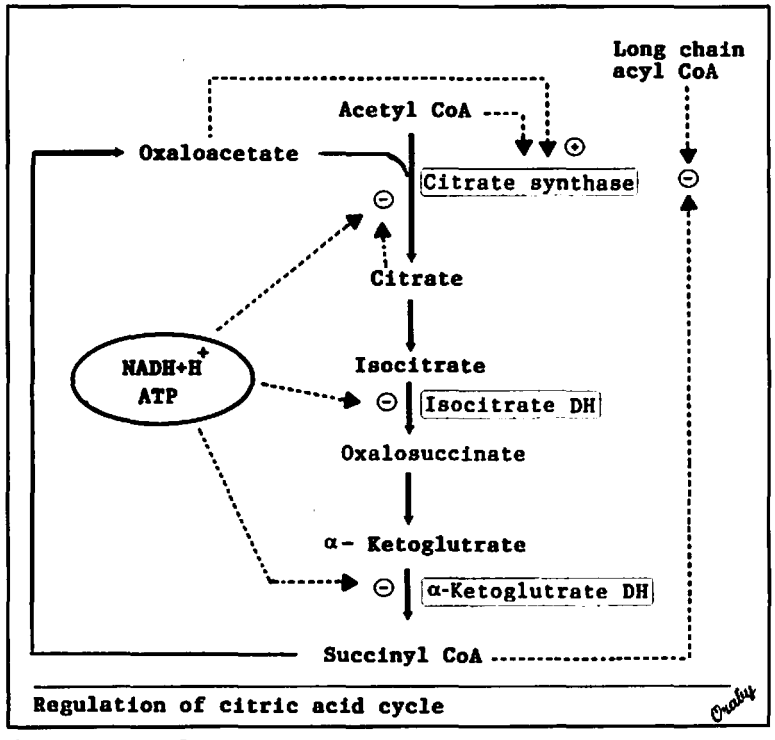
5) CO_2 produced in TCA is used in the following (CO_2 **fixation**) reactions:

- Pyruvate + CO_2 ➔ Oxaloacetate —Gluconeogenesis→ Glucose.
- Acetyl COA + CO_2 ➔ Malonyl CoA ➔ Fatty acids.
- Ammonia + ATP + CO_2 ➔ Carbamoyl phosphate ➔ Urea and pyrimidine.
- Propionyl CoA + CO_2 ➔ Methyl malonyl CoA ➔ Succinyl CoA ➔ Intermediate of citric acid cycle.
- Formation of C_6 of purine.
- Synthesis of H_2CO_3 / HCO_3 buffer.

7. **In vitro inhibition of TCA cycle :**
   a) **Flouroacetate** (F_1-CH_2-COSCoA): inhibits aconitase enzyme.
   b) **Arsenate:** inhibits α-ketoglutarate dehydrogenase enzyme.
   c) **Malonic acid**: inhibits succinate dehydrogenase enzyme (competitive inhibition).



8. **Regulation of citric acid cycle:**
   TCA is regulated through the **key enzymes** (citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase) and the **availability of O_2:**



a) Citrate synthase :
   1) Stimulated by acetyl CoA, oxaloacetate, ADP and NAD^+.
   2) Inhibited by long chain acyl CoA, citrate, succinyl CoA, ATP and NADH, H^+.

b) Isocitrate dehydrogenase and α-ketoglutarate dehydrogenase:

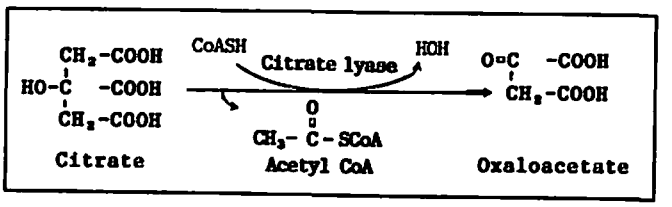
1) Stimulated by NAD^+, ADP.
2) Inhibited by NADH, H^+ and ATP.

c) **Availability of Oxygen:** Citric acid cycle needs oxygen to proceed (i.e. aerobic pathway). This is because in absence of oxygen, respiratory chain is inhibited leading to increase $NADH,H^+$ ➔ inhibition of TCA cycle.

9. Sources and fate of oxaloacetate:

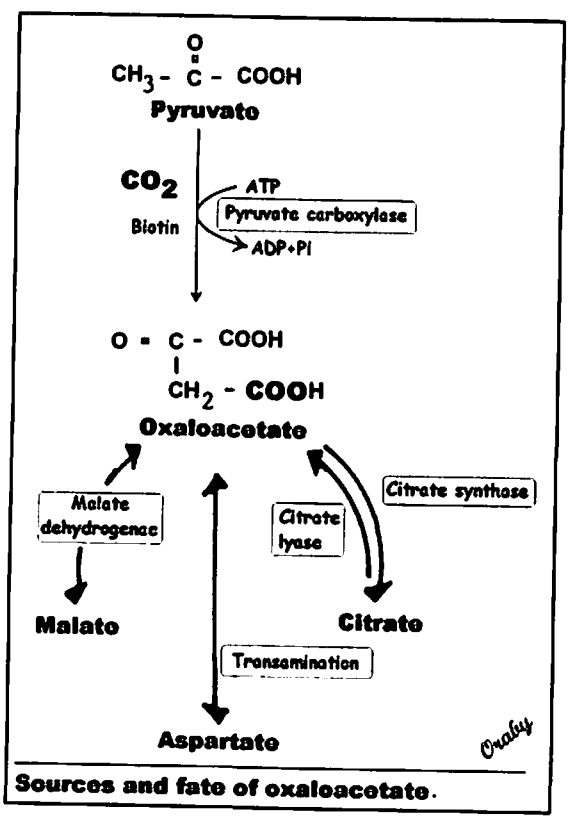
a) Sources of oxaloacetate:

1) **Oxidation of malate:** Final step in TCA cycle.
2) **Transamination of aspartate:** See protein metabolism.
3) **Carboxylation of pyruvate:** By pyruvate carboxylase and biotin (see gluconeogenesis).
4) **Cleavage of citrate:**



b) Fate of oxaloacetate:

1) **Formation of citrate:** By citrate synthase (first step in TCA cycle).
2) **Reduction to malate.**
3) **Transamination into aspartic acid.**



Sources and fate of oxaloacetate.

10. Energy production at substrate level in glucose oxidation:

a) The removal of hydrogen atoms from a compound is accompanied by a release of energy. If this energy is captured in phosphate or sulfate bonds, it will produce high-energy compounds.

b) The high energy compounds formed by glucose oxidation are:

1) Glyceraldhyde-3-p → 1,3 BPG (phosphate bond).
2) Phosphoglycerate → phosphoenol pyruvate (phosphate bond).
3) Pyruvate → Acetyl CoA (sulfate bond).
4) α-Ketoglutarate → Succinyl CoA (sulfate bond).

11. Pasteur effect:

a) It is the inhibition of glycolysis (anaerobic oxidation) by the presence of oxygen.

b) **Explanation:** Aerobic oxidation of glucose produces increased amount of ATP and citrate → those inhibit phosphofructokinase-1 (one of the key enzymes of glycolysis) → Inhibition of glycolysis.

III. *Pentose Phosphate Pathway (Hexose Phosphate Pathway):*

A. **Definition:** It is an alternative pathway for glucose oxidation where:

1. ATP (**energy**) is neither produced nor utilized.
2. Its main function is to produce **NADPH ,H^+** and **pentoses.**

B. **Location:**

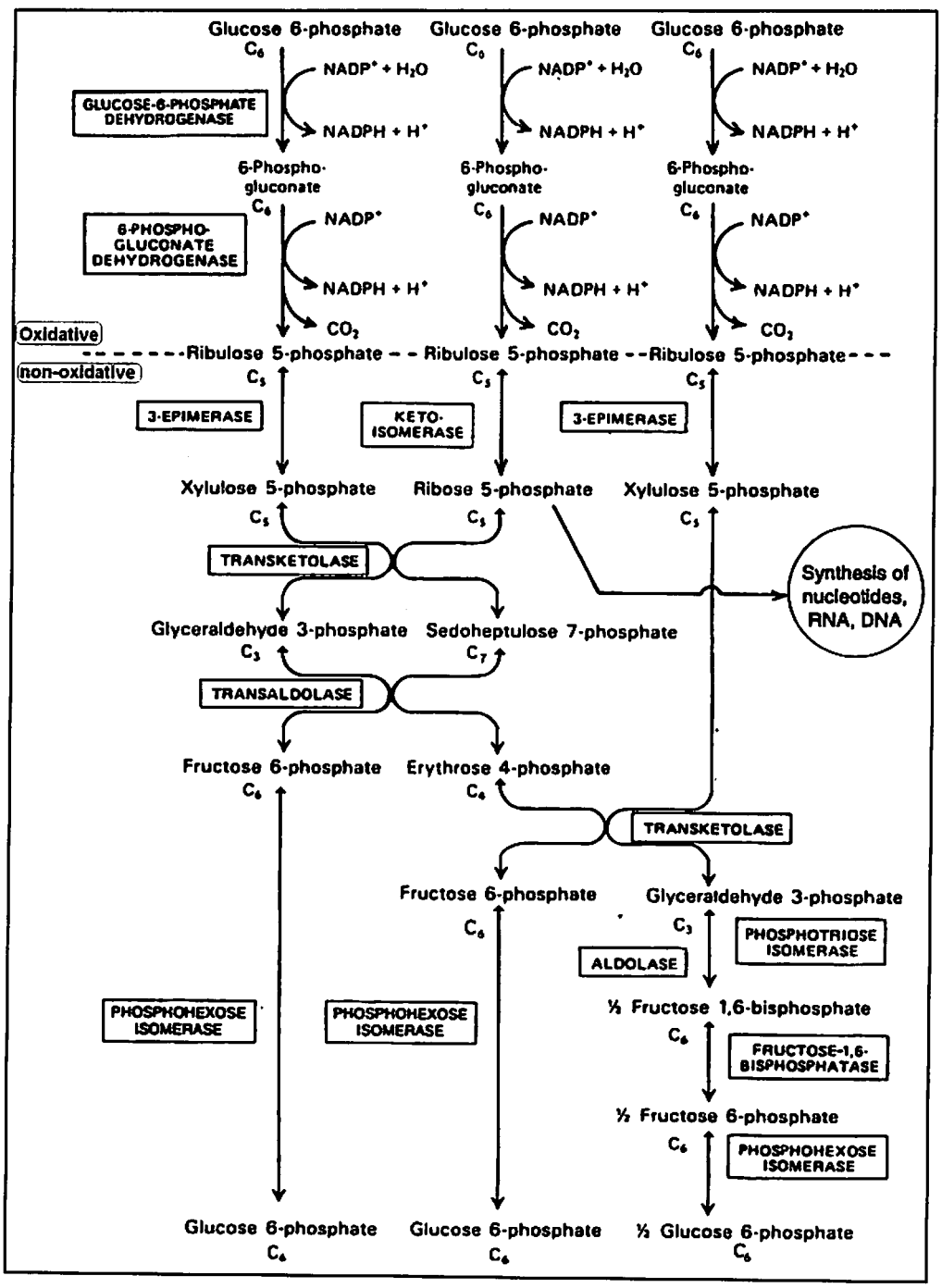
1. **Intracellular location:** cytosol.
2. **Organ location:**
   a) It is active in tissues where **NADPH,H^+** is needed for fatty acids and steroids synthesis.
      1) **Adipose tissue and liver:** It supplies NADPH,H^+ for Lipogenesis.
      2) **Adrenal cortex, ovaries and testes: It** supplies NADPH, H^+ for steroid synthesis.
      3) **Red cells: It** supplies NADPH,H^+ for production of reduced glutathione.
      4) **Retina:** It supplies NADPH,H^+ for reduction of retinal into retinol.

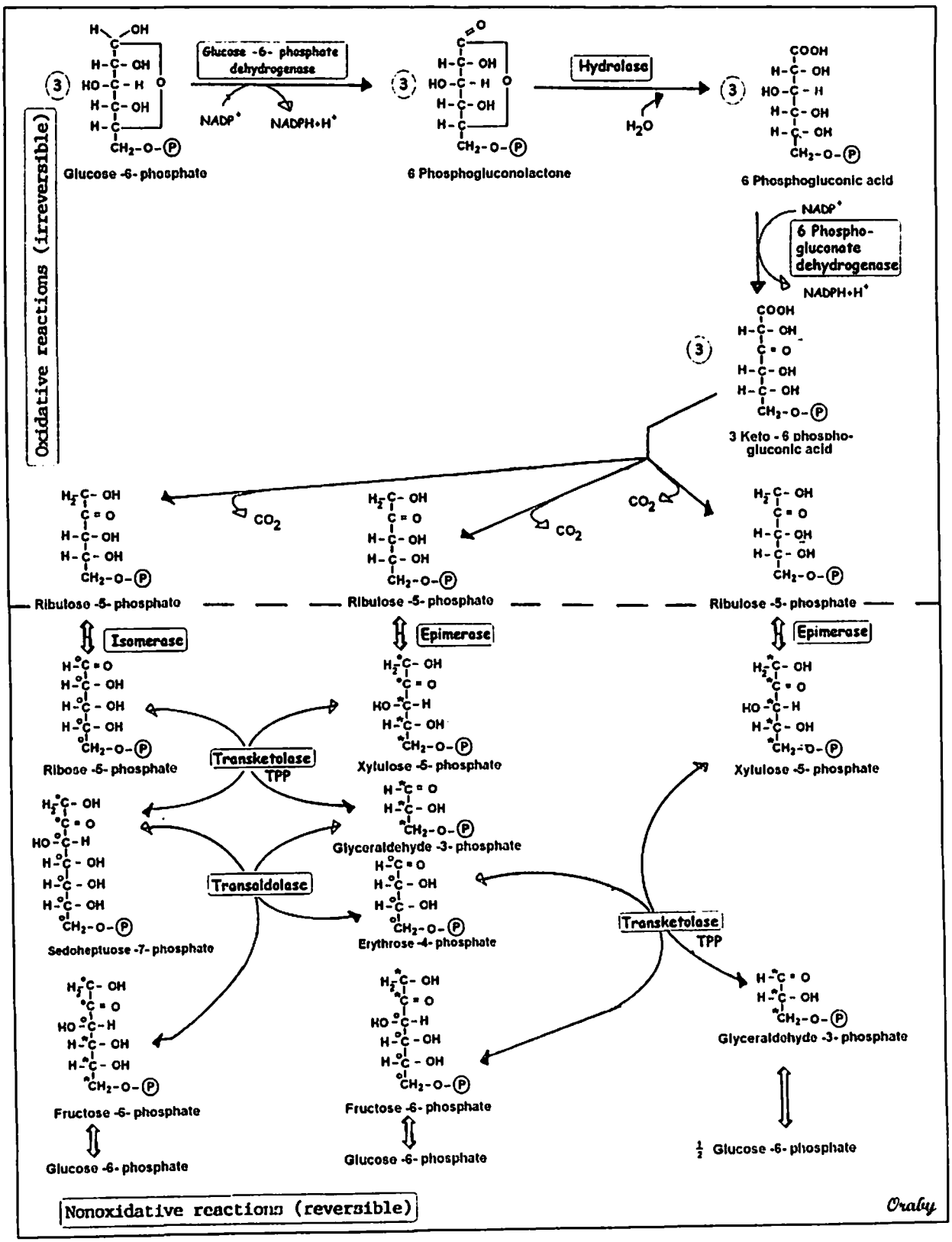
b) **In many tissues:** It supplies **pentoses** for synthesis of nucleotides.

C. Reactions (steps):

This pathway occurs in two phases; oxidative and non-oxidative:

1. **Oxidative (irreversible) phase:** where 3 molecules of glucose-6-phosphate are converted into 3 molecules of ribulsose-5-phosphate with production of $NADPH,H^+$ and CO_2 .
2. **Non-oxidative (reversible) phase:** Where the 3 molecules of ribulose-5-phosphate are interacted and converted into 2 molecules of glucose-6-phosphate and one molecule of glyceraldhyde-3-phosphate.




Oxidative reactions (irreversible)
Glucose -6- phosphate
Glucose -6- phosphate dehydrogenase
NADP+
NADPH+H+
6 Phosphogluconolactone
Hydrolase
H2O
6 Phosphogluconic acid
6 Phospho-gluconate dehydrogenase
3 Keto - 6 phospho-gluconic acid
CO2
Ribulose -5- phosphate
Isomerase
Epimerase
Ribose -5- phosphate
Xylulose -5- phosphate
Transketolase
TPP
Transaldolase
Glyceraldehyde -3- phosphate
Sedoheptuose -7- phosphate
Erythrose -4- phosphate
Fructose -6- phosphate
½ Glucose -6- phosphate
Nonoxidative reactions (reversible)
Oraby

D. Functions of pentose phosphate pathway:

Production of pentoses: ***(which are constituent of)***

- *RNA ,DNA .*
- *ATP ,GTP……..etc.*
- *NAD^+ ,FAD……etc.*

Production of NADPH + H^+: ***(which is necessary for)***

- *synthesis of substrates e.g. FA , cholesterol…….etc.*
- *Reduction of glutathione.*
- *Hydroxylation of aromatic compounds.*
- *Phagocytosis and respiratory burst.*

1. Production of pentoses:

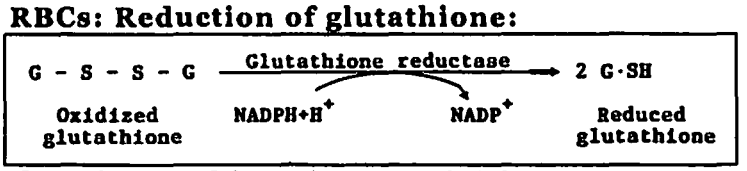
Which are essential for synthesis of nucleic acids (RNA and DNA), nucleotides (as ATP, GTP) and coenzymes (as NAD^+, NADP, FAD).

2. Production of NADPH, H^+: It is important for:

a) Synthesis of many substrates:

1) Synthesis of **fatty acids** (lipogenesis) cholesterol and other steroid hormones.
2) Synthesis of **sphingosine** and **galactolipids.**
3) Essential for **glucuronic acid** metabolism.
4) Synthesis of **non essential amino acids.**
5) Synthesis of **malate** from pyruvate by malic enzyme.

b) In RBCs: Reduction of glutathione:



Reduced glutathione is essential for:

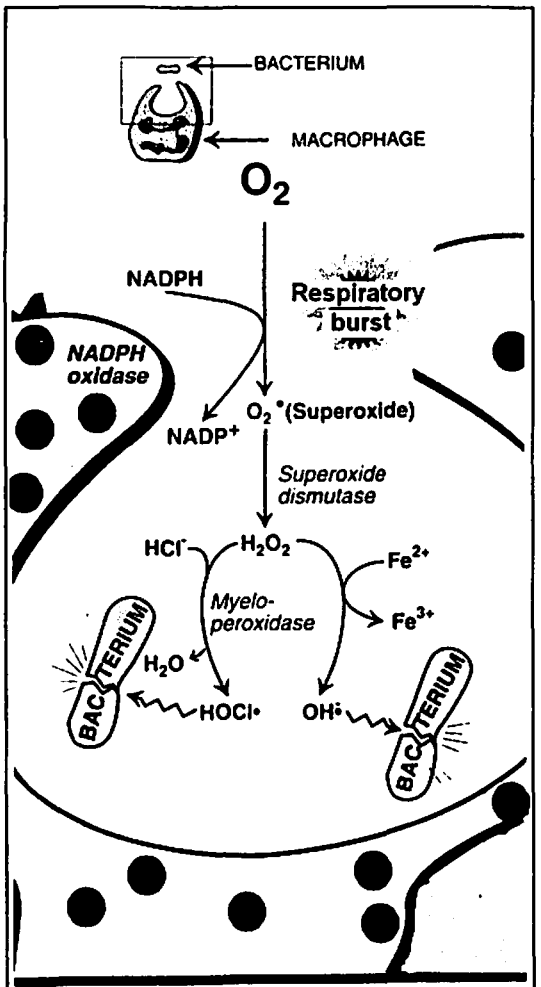
1) Normal **integrity of RBCs.**
2) Maintenance of **SH group** of RBCs enzymes.
3) Removal of **hydrogen peroxide (H_2O_2),** which is a toxic compound that causes cell membrane fragility.
4) Inactivation of insulin
5) Detoxication of many drugs and carcinogens

c) In liver: Hydroxylation of aromatic and aliphatic compounds:

NADPH,H^+ acts as coenzyme for **liver microsomal P-450 mono-oxygenase system (enzyme).** This is the major pathway for the **hydroxylation** of toxic aromatic and aliphatic compounds such as steroids, alcohols and many drugs converting them into non-toxic compounds (see chapter of xenobiotics, part I).

d) Phagocytosis by white blood cells (respiratory burst):

1) Phagocytosis is the engulfment of microorganisms and foreign bodies by white blood cells.
2) White blood cells contain an enzyme called: **NADPH+H⁺ oxidase enzyme** present in cell membrane.
3) After phagocytosis has occurred, NADPH+H⁺ oxidase converts oxygen, O_2 (derived from surrounding tissues) into super oxide ions (O_2^-).



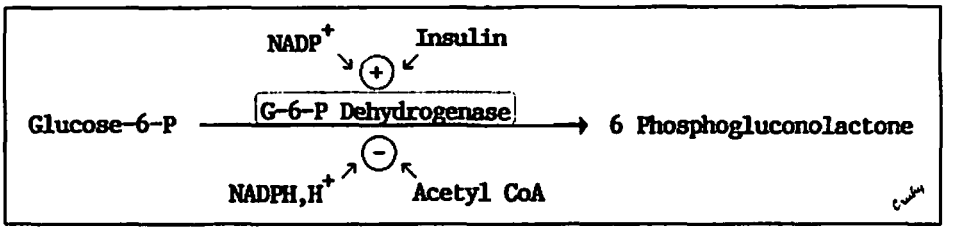
Neutrophil phagocytosis and the oxygen-dependent *myeloperoxidase* system for killing bacteria in phagolysosomes.

4) **Definition of respiratory burst:**
It is the rapid consumption of molecular oxygen that accompanies the formation of superoxid.
5) Superoxide is then converted into H_2O_2 by **superoxide dismutase** enzyme.
6) H_2O_2 by myeloperoxidase enzyme in the presence of HCl is converted into hypochlorite ($HOCl^-$), which kills bacteria.

e) **Deficiency of NADPH+H⁺-oxidase** leads to chronic bacterial infection.

E. **<u>Regulation of Pentose phosphate pathway</u>:**

Glucose-6-phosphate dehydrogenase is the key enzyme of pentose phosphate pathway. It is stimulated by insulin and NADP⁺ and inhibited by NADPH, H⁺ and acetyl CoA.

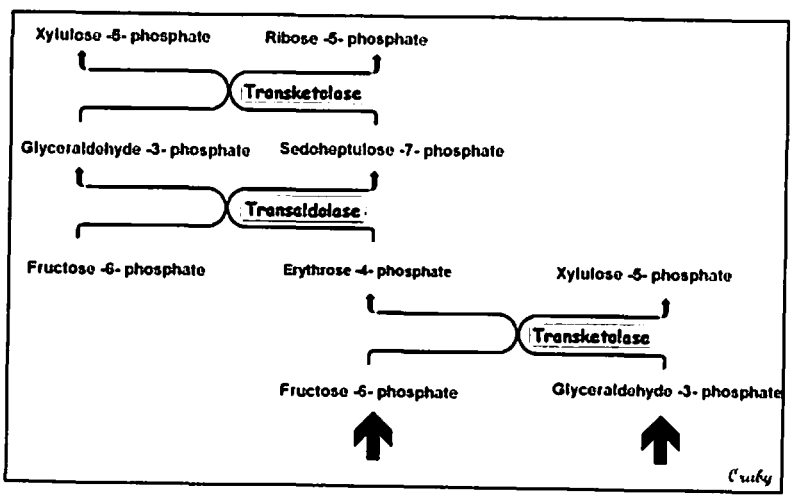


F. **Differences between pentose phosphate pathway (PPP) and glycolysis:**

	PPP	Glycolysis
Location	In certain cells	In all cells
Oxidation of glucose	Oxidation occurs in the first reaction.	Phosphorylation occurs first then oxidation
Coenzyme	$NADP^+$	NAD^+
Energy	No energy production	2 or 8 ATP
CO_2	Produced	Not produced
Pentoses	Produced	Not produced

G. **Pentose phosphate pathway in skeletal muscles:**

1. Skeletal muscles are poor in glucose-6-phosphate dehydrogenase enzyme, but they contain transketolase and transaldolase enzymes.
2. Skeletal muscles obtain their pentose requirement by reversible reactions of pentose phosphate pathway, using fructose-6-phosphate and glyceraldehyde-3-p and the enzymes transketolase and transaldolase.



H. **Defects of pentose phosphate pathway:**

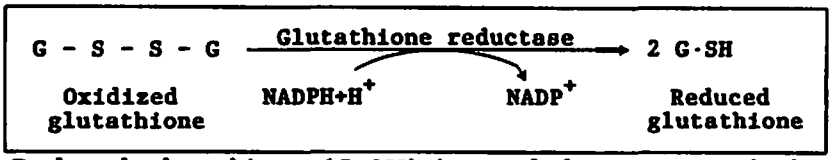
Favism (Deficiency of glucose-6-phosphate dehydrogenase enzyme):

1. Definition:

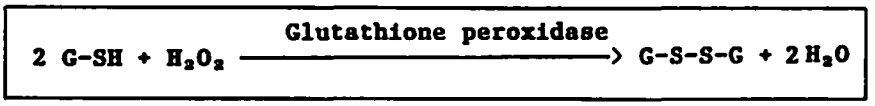
It is type of a hemolytic anemia (excessive destruction of RBCs) results after ingestion of fava beans and some other compounds. These compounds alter the structure of the enzyme → ↓ G-6-P dehydrogenase enzyme activity.

2. Mechanism:

a) Deficiency of glucose-6-P dehydrogenase ➔ Decreased NADPH,H⁺ production (which is essential to reduce glutathione in RBCs).



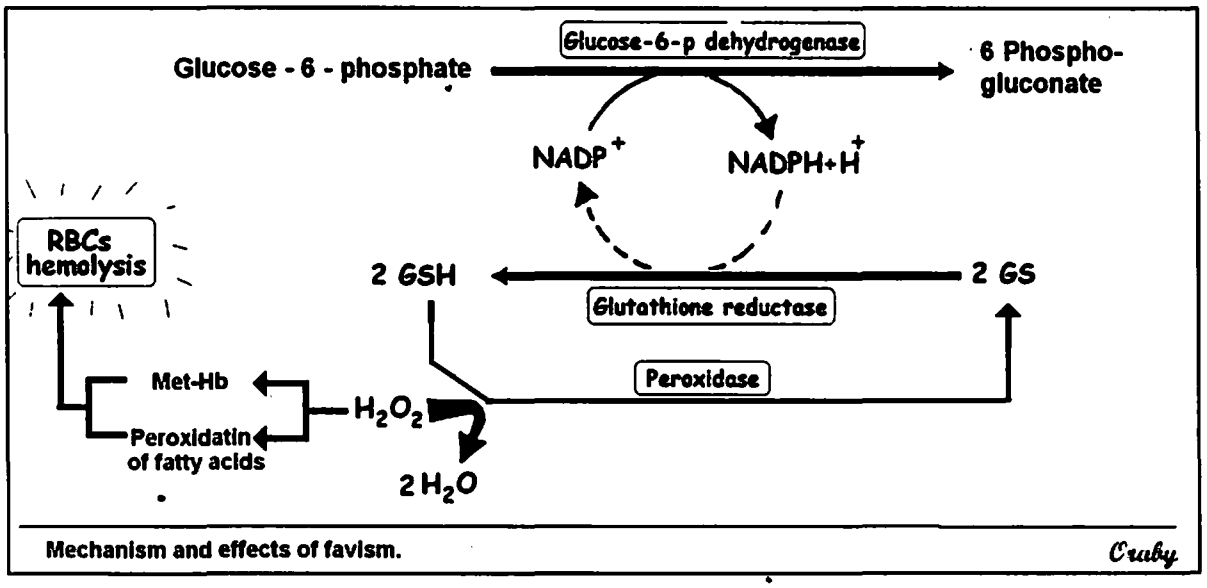
b) Reduced glutathione (G-SH) is needed to remove hydrogen peroxide (H_2O_2) which is toxic to the cell.



Deficiency of glucose-6-P DH → ↓ NADPH,H⁺ ➔ ↓ reduced glutathione ➔ Accumulation of H_2O_2 ➔ Hemolysis of RBCs

c) Effect of H_2O_2 on RBCs:

1) H_2O_2 causes peroxidation of fatty acids present in cell membrane ➔ Hemolysis.
2) H_2O_2 causes conversion of hemoglobin into met-hemoglobin. These toxic compounds increase the red cell membrane fragility.



Mechanism and effects of favism. Ruby

3. Signs and symptoms of favism:

a) Patients with enzyme deficiency show attacks of hemolytic anemia in the form of severe jaundice and decreased hemoglobin concentration when exposed to certain oxidizing agents such as:

1) **Special food** as fava beans.
2) **Antimalarial drugs:** as primaquine.
3) **Antibiotics** as streptomycin and sulfa.

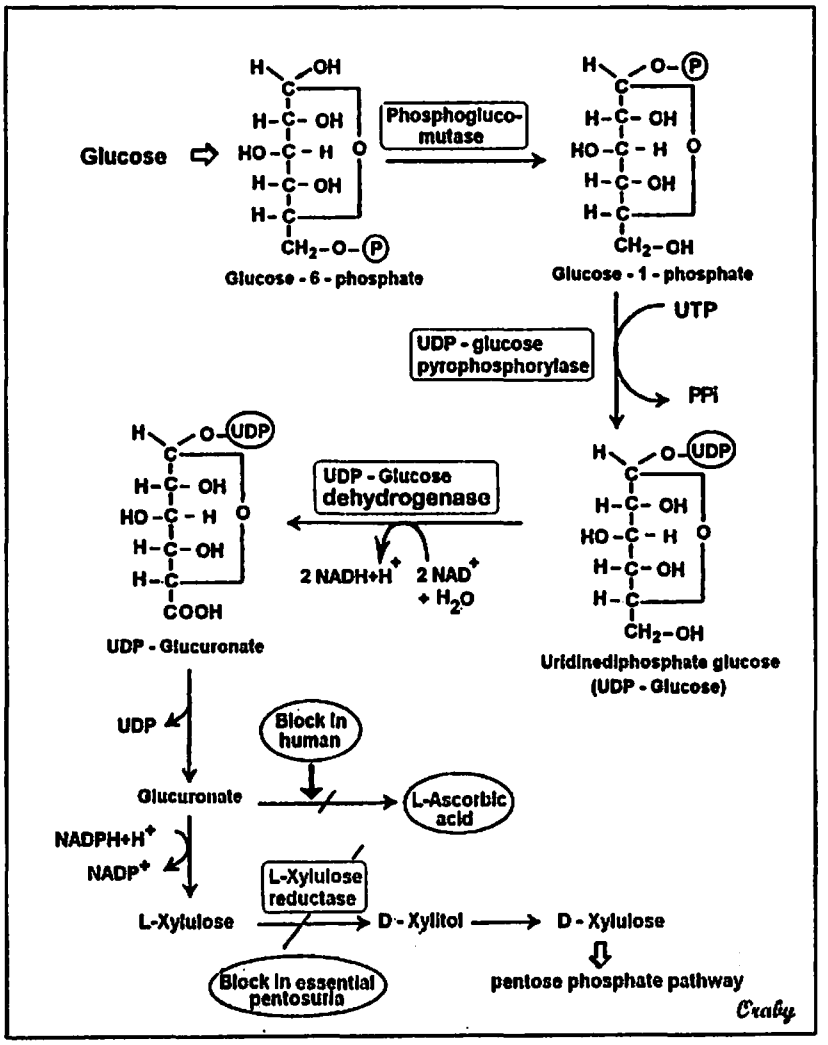
IV. *Uronic acid pathway:*

A. **Definition:** It is a minor pathway, in which glucose is converted into glucuronic acid.

B. **Location of the pathway:**

1. **Intracellular location:** cytosol.
2. **Organ location:** Mainly liver.

C. **Steps:**



D. **Functions (importance) of uronic acid pathway:**

This pathway produces **glucuronic acid**, which is important for:

1. **Synthesis of substrates:**
   a) Glycosaminoglycans.
   b) Vitamin C, L-ascorbic acid ***(not in human)***.

2. **Conjugation reactions:**
   UDP-glucuronic acid is used for conjugation with many body compounds to make them more soluble before excretion e.g. steroid hormones and bilirubin.
3. **Detoxification reactions:**
   UDP-glucuronic acid is used for conjugation with toxic compounds to make them less toxic e.g. phenols (see chapter of xenobiotics, part I).

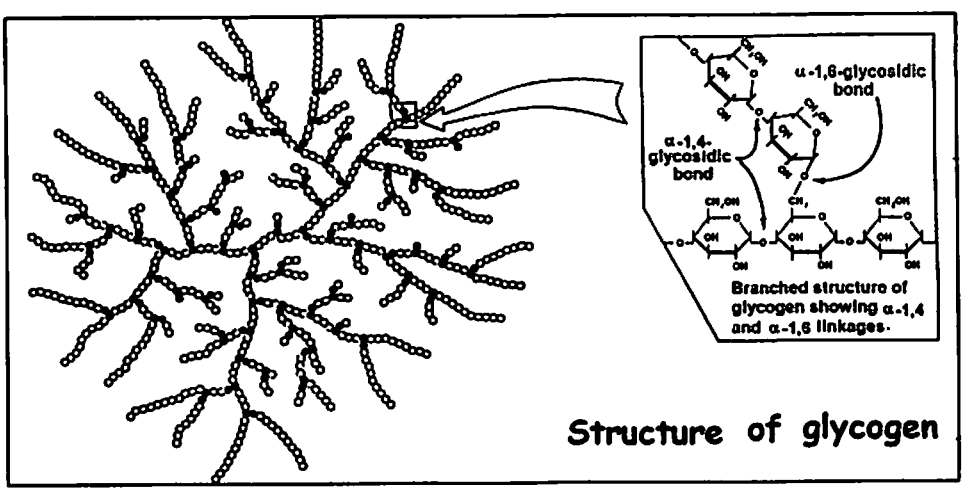
E. **Fate of glucuronic acid** (see diagram):
UDP-glucuronate is converted to glucuronate then → L-xylulose → D-xylitol → D-xylulose → which then joins pentose phosphate pathway to be completely oxidized.

F. **Defects of uronic acid pathway = Essential pentosuria:**
1. It is ***benign*** rare hereditary disease due to failure of conversion of L-xylulose into D-xylulose (due to **deficiency of L-xylulose reductase).**
2. L-xylulose will accumulate and be excreted in urine. Subjects excrete 1 to 4 grams of L-xylulose in the urine each day.

Glycogen Metabolism

1. *Structure of glycogen:*

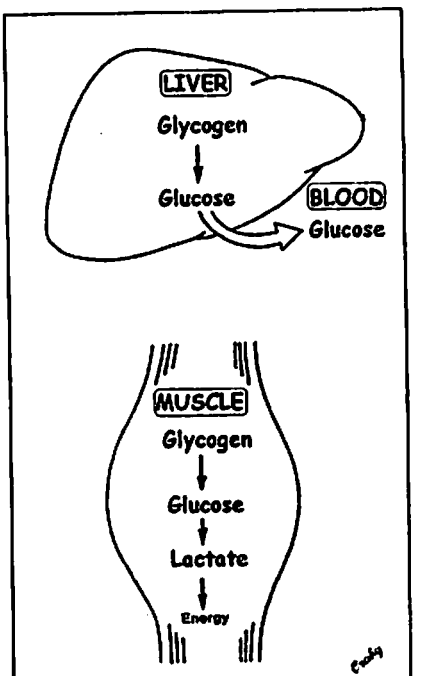
A. Glycogen is homopolysaccharide formed of branched α D glucose units (α 1,4 and α 1,6).
B. The main glycosidic bond is α1-4-linkage. Only at the branching point, the chain is attached by α1-6 linkage.
C. Each branch is made of 12-14 glucose units.



II. ***Location of glycogen:*** Glycogen is present mainly in cytosol of liver and muscles.

A. **Liver glycogen** is about 120 grams (about 6 % of liver weight).

B. **Muscle glycogen** is about 350 grams (about 1 % of total muscles weight).



Location and function of liver and muscle glycogen.

III. ***Functions of glycogen:***

A. **Liver glycogen:** It maintains normal blood glucose concentration especially during the early stage of fast (between meals). After **12-18 hours** fasting, liver glycogen is depleted.

B. **Muscle glycogen:** It acts as a source of energy within the muscle itself especially during muscle contractions.

IV. ***Synthesis of glycogen (glycogenesis):***

A. **Definition:** It is the formation of glycogen in liver and muscles.

B. **Substrates for glycogen synthesis:**

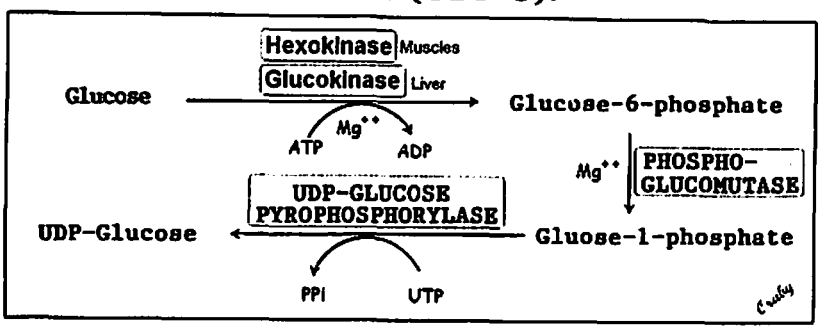
1. **In liver:**
   a) Blood glucose.
   b) Other hexoses: fructose and galactose.
   c) Non-carbohydrate sources: (gluconeogenesis) e.g. glycerol and lactate. These are converted first to glucose, then to glycogen.
2. **In muscles:**
   a) Blood glucose only.

C. **Steps:**

Glucose molecules are the first activated to uridine diphosphate glucose **(UDP-G)**. Then these UDP-G molecules are added to a glycogen primer to form glycogen.

$$\text{Glucose} \Rightarrow \text{UDP-Glucose} \xrightarrow[\text{Glycogen synthase, Branching enzyme}]{\text{Glycogen primer}} \text{Glycogen}$$

1. Formation of UDP-Glucose (UDP-G):



- **Note: Glucose is converted into glucose-6-phosphate by glucokinase in liver and hexokinase in muscles.**

2. Formation of glycogen:

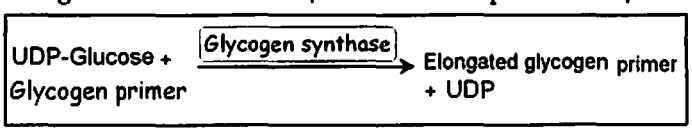
a) UDP-Glucose reacts with glycogen primer, which may be:

1) **Few molecules of glucose** linked together by α1-4 linkage.

2) **A protein called glycogenin.** UDP-G molecules react with -OH of tyrosine of that protein to initiate glycogen synthesis.

b) Glycogen synthase enzyme:

By the action of glycogen synthase (key enzyme of glycogenesis), UDP-G molecules are added to glycogen primer causing elongation of the α1-4 branches up to 12-14 glucose units.



c) Branching enzyme:

It transfers parts of the elongated chains (5-8 glucose residues) to the next chain forming a new α1-6 glycosidic bond. The new branches are elongated by the glycogen synthase and the process is repeated.

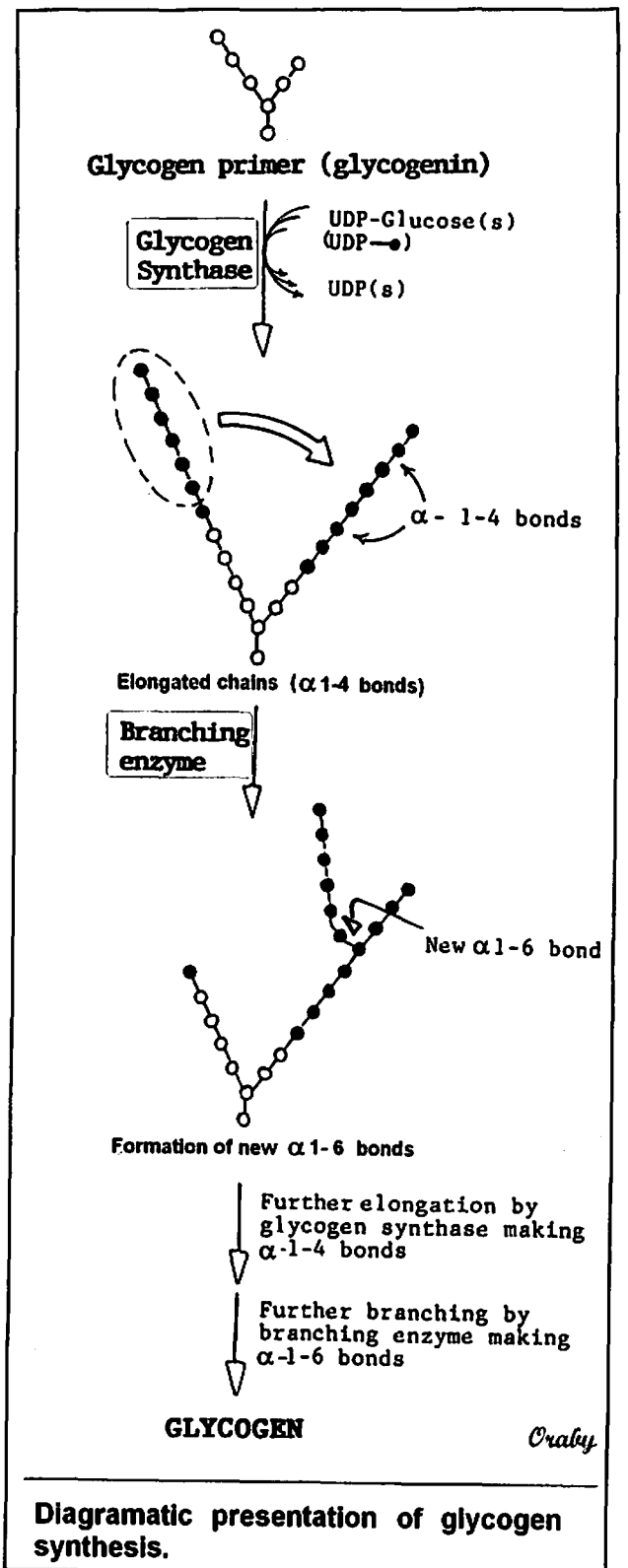
V. *Breakdown of glycogen (Glycogenolysis):*

A. Definition:

It is the breakdown of glycogen into glucose (in liver) and lactic acid (in muscles).

B. Steps:

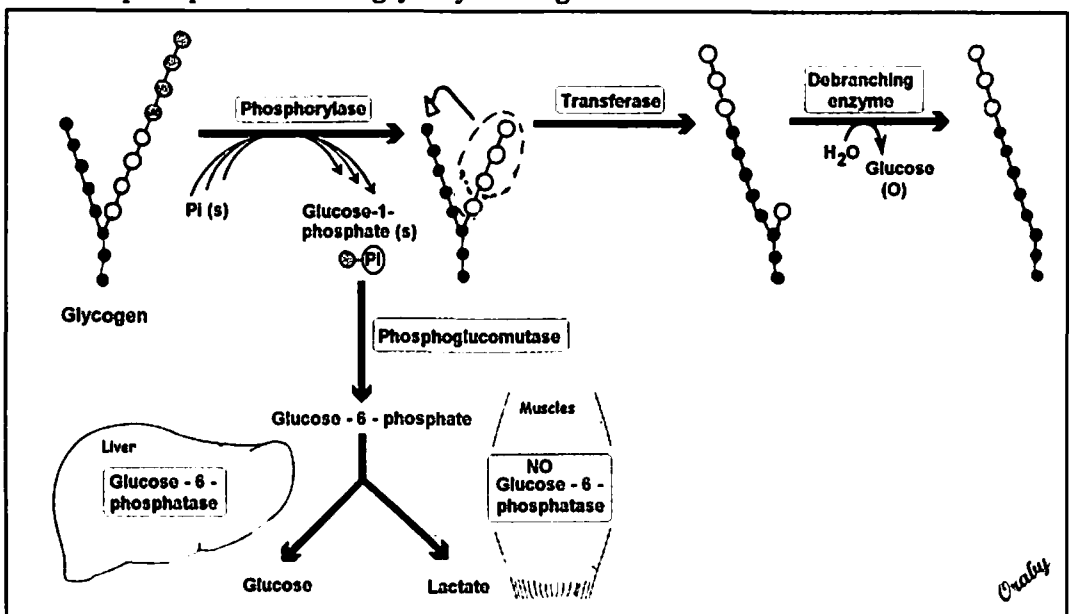
1. **Phosphorylase** (the key enzyme of glycogenolysis) acts on α1-4 bonds, breaking it down by phosphorolysis (i.e. breaking down by addition of inorganic phosphate "Pi"). Therefore, it removes glucose units in the form of glucose-1-phosphate.



Diagramatic presentation of glycogen synthesis.

2. Phosphorylase enzyme acts on the branches containing more than 4 glucosyl units.
3. When the branch contains 4 glucose units, 3 of them are transferred to a next branch by transferase enzyme, leaving the last one.

4. The last glucose unit that is attached to the original branch by α1-6 bond is removed by debranching enzyme by hydrolysis (i.e. breaking the bond down by addition of H_2O).
5. Glucose-1-phosphate molecules are converted to glucose-6-phosphate, by mutase enzyme.
6. **Fate of glucose-6-phosphate:**
   a) **In liver:** glucose-6-phosphate is converted to glucose by glucose-6-phosphatase.
   b) **In muscles:** there is no glucose-6-phosphatase, so glucose-6-phosphate enters glycolysis to give lactate.



Mechanism of glycogenolysis in liver and muscles.

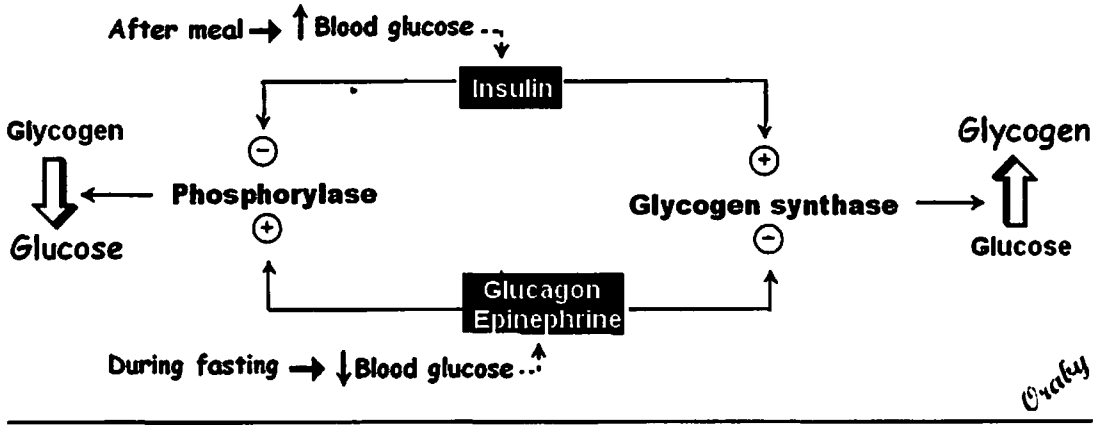
VI. *Regulation of glycogenesis and glycogenolysis:*

A. There is a **coordinated** regulation of glycogenesis and glycogenolysis i.e. conditions leading to stimulation of glycogenolysis, inhibiting at the same time glycogenesis and vise versa.

B. **During fasting,** glycogenolysis is stimulated and glycogenesis is inhibited. This provides blood glucose from liver glycogen.

C. **After meal,** part of absorbed glucose (40%) goes to general circulation to be utilized. The remaining (60%) is converted into glycogen in liver. So after meal, glycogenesis is stimulated and glycogenolysis is inhibited.

D. **The principle enzymes** controlling glycogen metabolism are glycogen **synthase** and **phosphorylase**. These are regulated as follows:



Summary of hormonal regulation of glycogenesis and glycogenolysis.

1. During fasting:

Glycogenolysis is stimulated by:

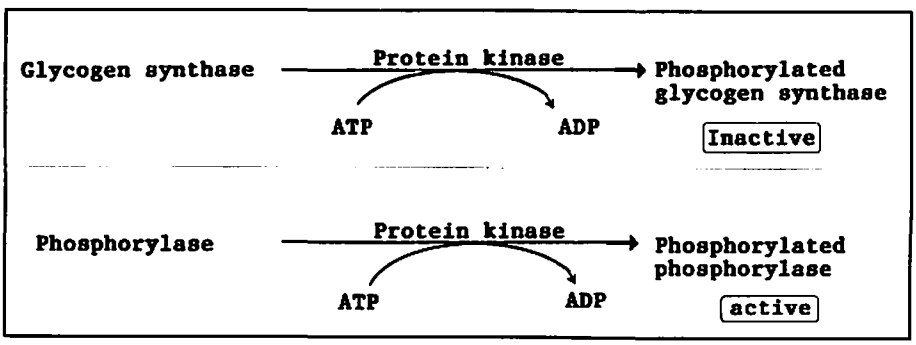
* Cyclic AMP and protein kinase
* Calcium ions and calmodulin protein

a) Blood glucose level tends to be decreased. This stimulates secretion of epinephrine, nor-epinephrine and glucagon hormones .

b) These hormones stimulate adenylate cyclase enzyme, which converts ATP into cyclic AMP (cAMP).

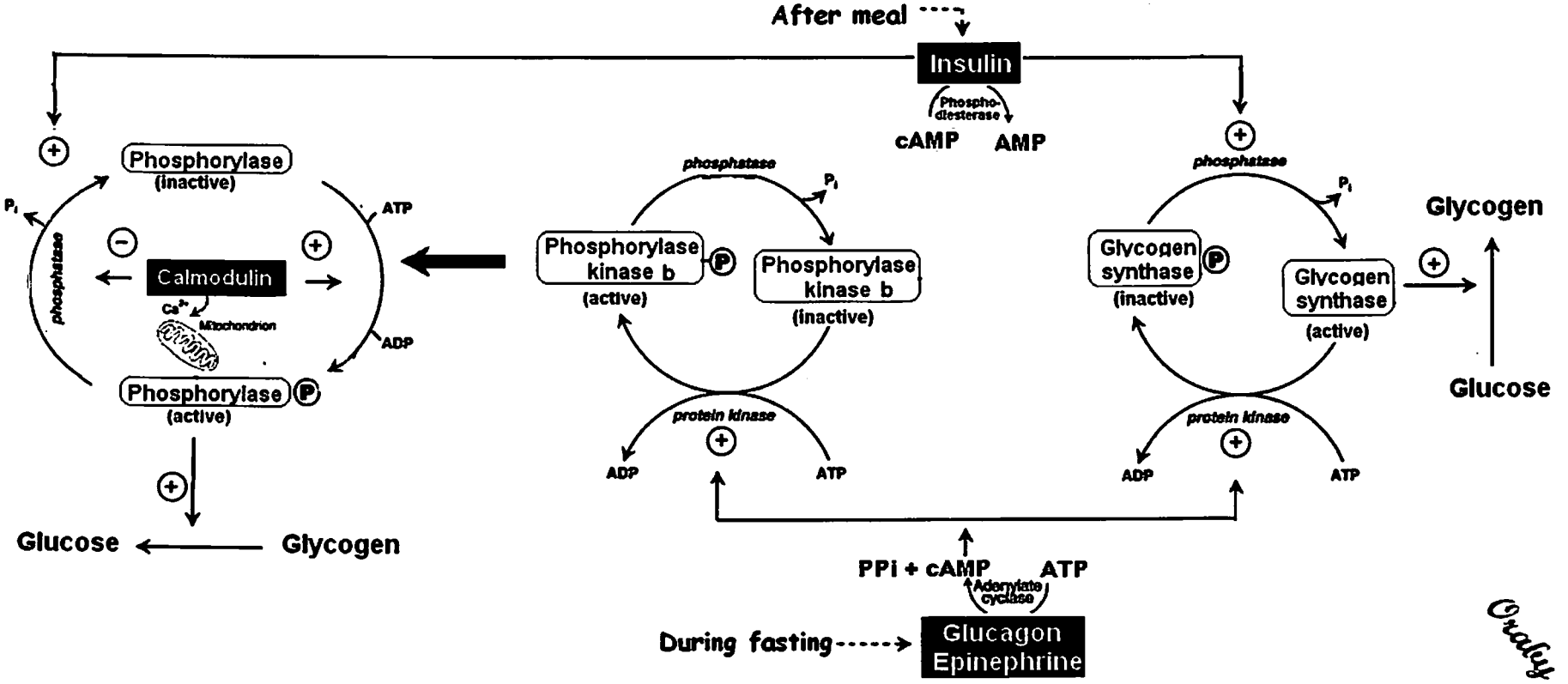
c) cAMP stimulates protein kinase enzyme .

d) Protein kinase enzyme stimulates phosphorylation of both glycogen phosphorylase and glycogen synthase.



e) As a result, glycogenolysis proceeds causing increase of blood glucose. At the same time , glycogenesis will be inhibited.

f) Epinephrine and nor epinephrine stimulate mobilization of calcium ions (Ca^{2+}) from mitochondria to cytosol. Ca^{2+} ions then combine with a protein called calmodulin causing its conformational changes and activating it.



Hormonal regulation of glycogenesis and glycogenolysis.

g) **The active calmodulin causes** phosphorylation of both glycogen phosphorylase and synthase (like protein kinase). This leads to stimulation of glycogenolysis.

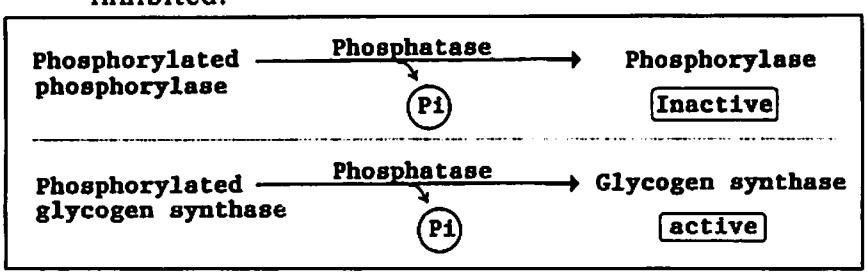
2. **After meal:**

a) Blood glucose level tends to be increased. This stimulates secretion of insulin hormone.

b) **Insulin causes the following:**

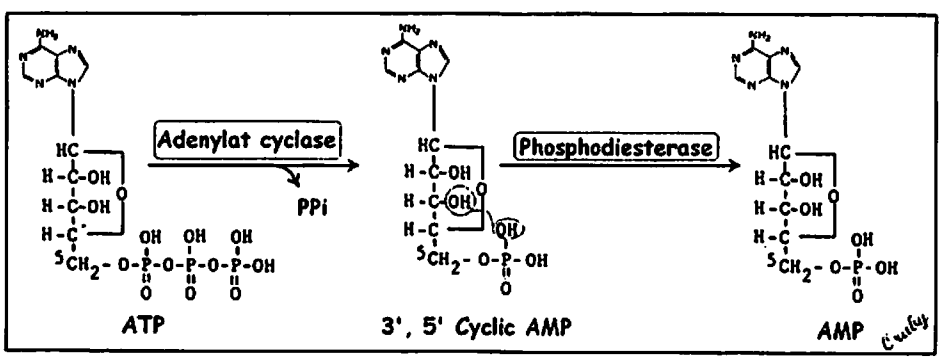
1) Stimulation of phosphodiesterase enzyme, which converts, cAMP into AMP i.e. abolishes the stimulatory effect of cAMP.

2) Stimulation of phosphatase enzyme, which removes phosphate from phosphorylase (inhibiting it) and glycogen synthase (stimulating it). As a result glycogenesis will proceed and glycogenolysis will be inhibited.

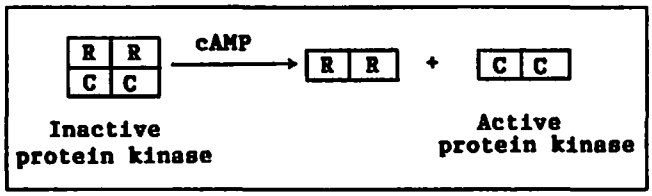


Notes:

1. **cAMP** is an intracellular compound , formed from ATP by an enzyme called : **adenylate cyclase** and destroyed by an enzyme called **phosphodiesterase** enzyme.



2. **Protein kinase** is an enzyme causing phosphorylation of substrate using ATP as a source of phosphate. **This enzyme is composed of 4 subunits:** 2 regulatory **(R)** and 2 catalytic **(C)**. The whole protein kinase is inactive, but binding of cAMP with it removes **(R)** subunits leaving the active catalytic subunit.



VII. *Differences between liver glycogen and muscle glycogen:*

	Liver glycogen	Muscle glycogen
Sources:	1-Blood glucose. 2-Other hexoses: e.g. fructose. 3-Non-carbohydrate sources : e.g. lactate	Blood glucose only.
Amount:	120 grams maximum	350 gram maximum
Concentration :	6%	1%
Functions:	It maintains normal blood glucose concentration between meals	private source of energy for muscles only
End product:	Glucose	Lactate (due to absence of glucose-6- phosphatase).
Effect of hormone:		
Insulin:	Stimulates glycogenesis	Same
Epinephrine:	Stimulates glycogenolysis	Same
Glucagon:	Stimulates glycogenolysis	**No effect**

VIII. *Glycogen storage diseases:*

A. Definition:

These are group of inherited disorders characterized by deposition of abnormal type or quantity of glycogen in the tissues.

B. Causes:

They are mainly due to deficiency of one of **enzymes of glycogen metabolism** e.g. glucose-6-phosphatase, debranching enzyme etc.

C. Types: (8 types):

1. Type one (I): Von Gierk`s disease:

a) It is due to deficiency of **glucose-6-phosphatase.**

b) It is the commonest type and characterized by:

1) Accumulation of large amount of glycogen in liver. This leads to **disturbance of liver functions.**

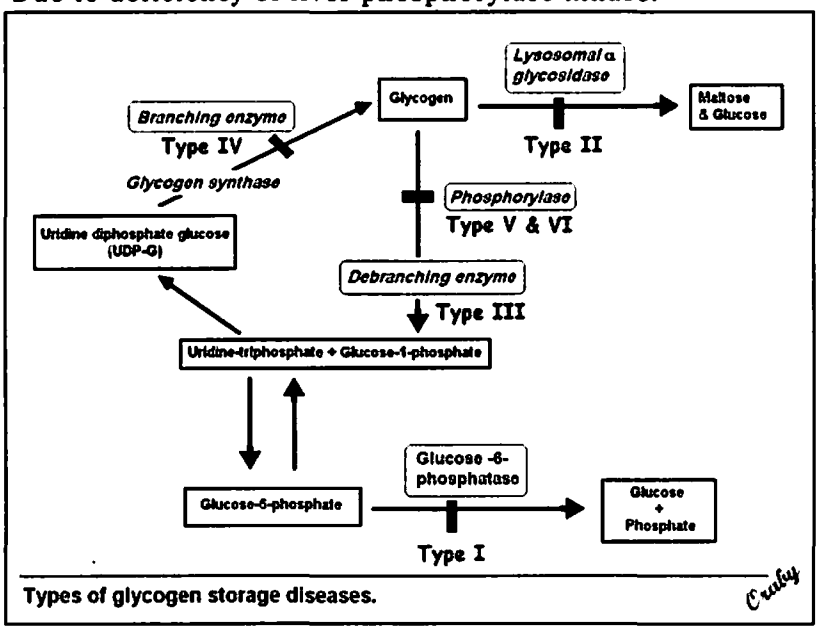
2) **Enlargment of liver** (hepatomegally).

3) **Fasting hypoglycemia.**

4) **Ketosis** and hyperlipidemia.

5) **Hyperuricemia (gout):** ↓ glucose-6-phosphatase ➔ ↑ Glucose-6-phosphate ➔ ↑ Pentose phosphate pathway ➔ ↑ Ribose production ➔ ↑ Uric acid ➔ Gout.

2. **Type two (II): Pompe`s disease:**
   a) Glycogen accumulates in lysosomes of all tissue cells.
   b) Normally, 1-3% of cellular glycogen is hydrolyzed by lysozomal glucosidase (acid maltase). Absence of this enzyme in lysosomes results in accumulation of glycogen in all tissues including heart. Death occurs during first year due to **heart failure.**
3. **Type three (III): Limit dextrosis (Cori`s disease):**
   a) Due to deficiency of **debranching enzymes** in liver, muscles and heart.
   b) Glycogen has many short branches.
4. **Type four (IV): Amylopectinosis:**
   a) It is due to absence of **branching enzyme.**
   b) Glycogen formed has no or few branches.
   c) Death due to cardiac or liver failure in first year of life.
5. **Type five (V): McArdle`s syndrome:**
   a) Due to deficiency of **muscle phosphorylase.**
   b) Glycogen is accumulated in muscles, and does not breakdown during exercise. This leads to decreased energy.
   c) **Muscle cramps** after short period of exercise.
6. **Type six (VI): Her`s disease :**
   a) Due to deficiency of **liver phosphorylase.**
   b) Glycogen is accumulated in liver.
   c) Fasting hypoglycemia.
7. **Type seven (VII): Tarui`s disease:**
   a) Due to deficiency of **phosphofructokinase** in muscle and RBCs.
   b) Hemolytic anemia and muscle cramps.
8. **Type eight (VIII):**
   a) Due to deficiency of liver phosphorylase kinase.



Types of glycogen storage diseases.

Gluconeogenesis

I. *Definition:*

Gluconeogenesis is the formation of glucose from non-carbohydrate sources. **These sources include:**

1. Lactate.
2. Pyruvate.
3. Glycerol.
4. Some amino acids.
5. Propionate (in ruminants only).

II. *Functions of gluconeogenesis:*

A. **Gluconeogenesis supplies the body with glucose:**

1. Glucose is the only source of energy for nervous tissues, RBCs and skeletal muscles during exercises.
2. Glucose is the precursor of milk sugar (lactose) in mammary gland.
3. Glucose is important during low carbohydrate diet or when liver glycogen is depleted (liver glycogen is depleted after 12-18 hours).

B. **Gluconeogenesis clears the blood** from the waste products of other tissues as lactate (produced by muscles and RBCs).

III. *Location of gluconeogenesis::*

A. **Intracellular location:** cytosol and mitochondria.

B. **Organ location:**

1. Liver (90%).
2. Kidney (10%).

IV. *Steps:*

The steps of gluconeogenesis are mainly the reversal of glycolysis, except for the three irreversible kinases which are replaced by the following enzymes:

Glycolysis	Gluconeogenesis
1. Glucokinase	1. Glucose-6-phosphatase
2. Phosphofructokinase- 1	2. Fructose 1,6 bisphosphatase
3. Pyruvate kinase	3. Pyruvate carboxylase
	4. Phosphoenol pyruvate carboxykinase

A. **Fructose 1,6 bisphosphate to fructose-6-phosphate:**

This reaction is catalyzed by the enzyme fructose 1,6 bisphosphatase.

B. **Glucose-6-phosphate to glucose:**

This reaction is catalyzed by the enzyme glucose-6-phosphatase.

C. **Pyruvate to phosphoenol pyruvate:**

1. This conversion is done by dicarboxylic acid shuttle and needs 2 enzymes:
   a) Pyruvate carboxylase: present in mitochondria.
   b) Phosphoenol pyruvate carboxykinase: present in cytosol.

2. Pyruvate should pass first from cytosol to mitochondria by special transporter.

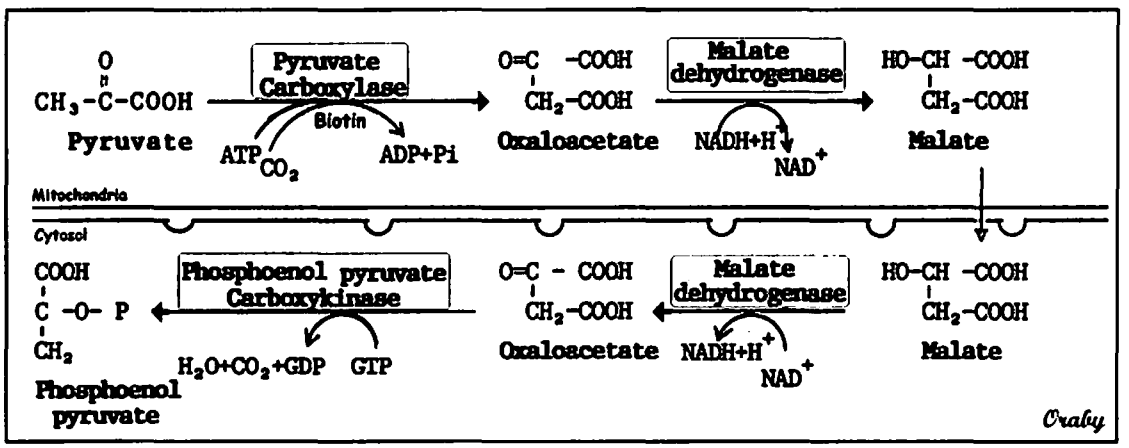
3. Pyruvate is then converted into oxaloacetate by pyruvate carboxylase (in the presence of biotin, CO_2 and ATP).

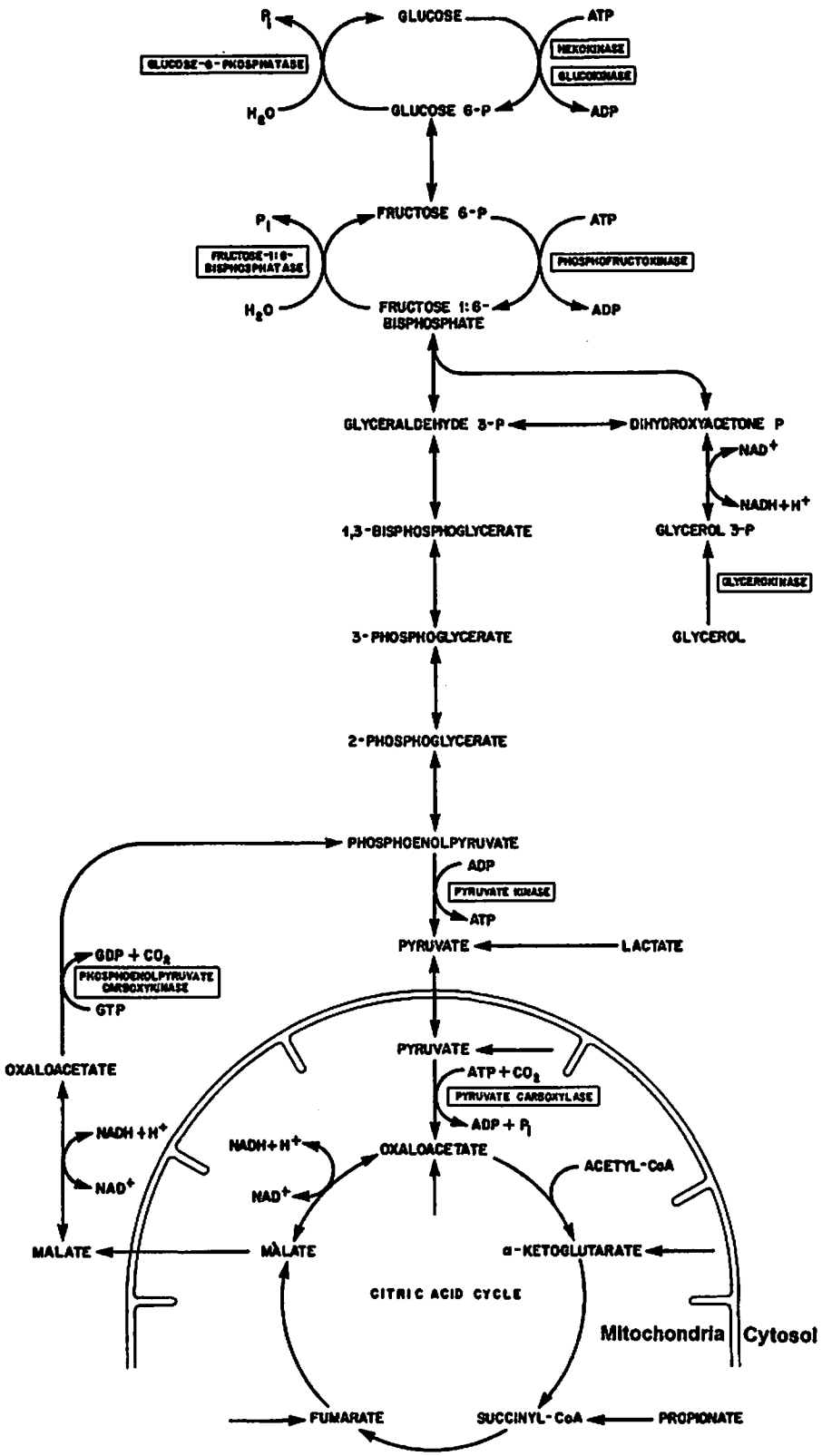
4. The mitochondrial membrane is impermeable to oxaloacetate. So oxaloacetate is converted to malate by malate dehydrogenase.

5. Malate is transported to cytosol, where it is converted again into oxaloacetate (by cytosolic malate dehydrogenase).

6. Oxaloacetate is converted into phosphoenol pyruvate by phosphoenolpyruvate carboxykinase (PEP).

Note: Pyruvate never goes in the course of citric acid pathway to reach malate, because this pathway needs insulin and other factors, which are deficient during gluconeogenesis.

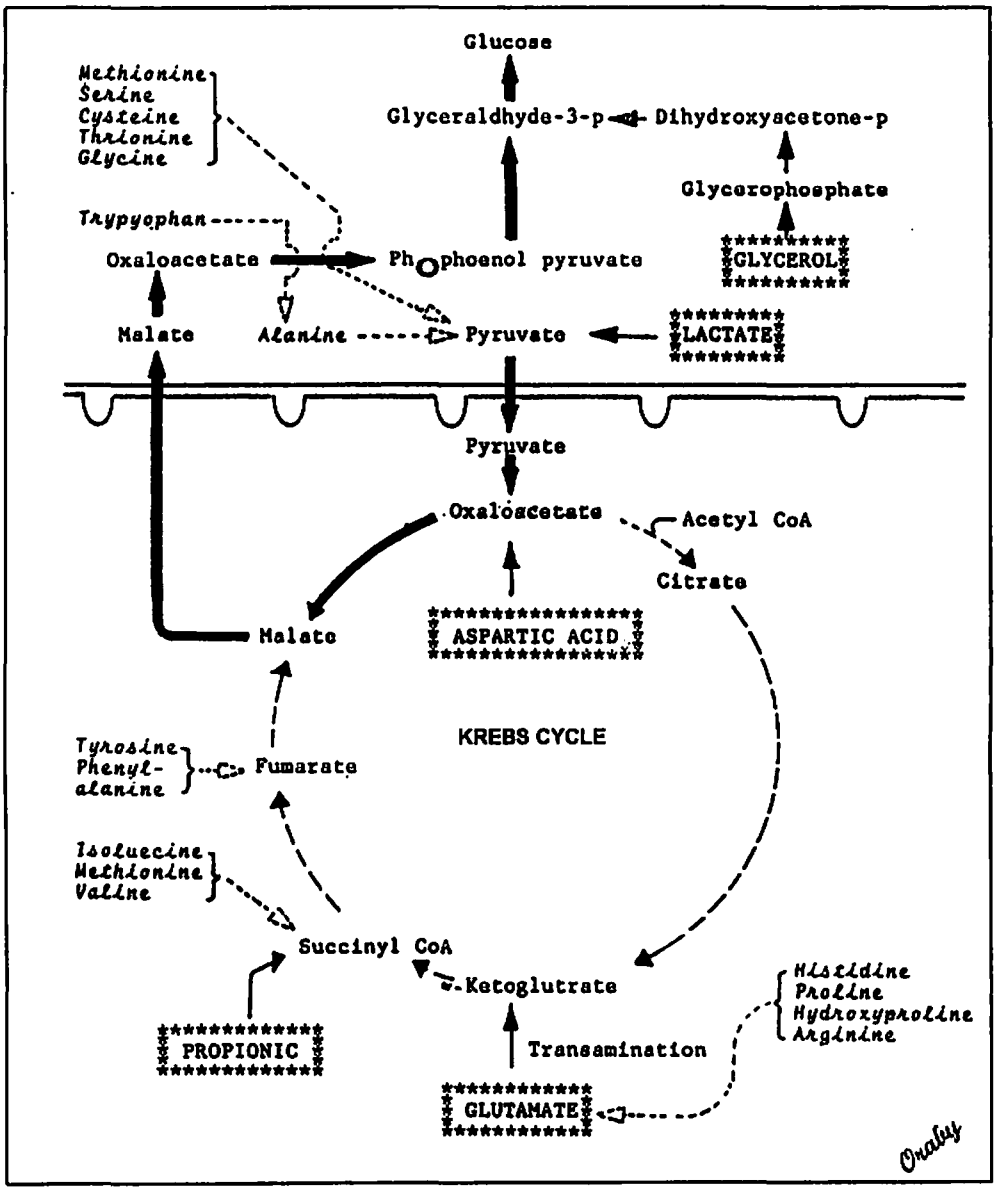




Major pathways in gluconeogenesis in the liver. Entry points of glucogenic amino acids after transamination are indicated by unlabeled arrows.

V. *Pathways for different sources of gluconeogenesis:*

Any substance that can join common pathway of gluconeogenesis is considered glycogenic.



A. Gluconeogenesis from lactate:

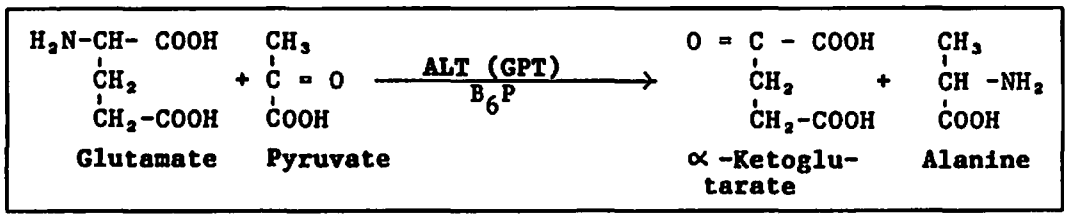
1. Lactate is converted into pyruvate by lactate dehydrogenase:

$$\text{Lactate} + NAD^+ \xrightarrow{\text{Lactate dehydrogenase}} \text{Pyruvate} + NADH + H^+$$

2. pyruvate then joins common pathway to give glucose (as indicated in the diagram).

B. Gluconeogenesis from glutamate:

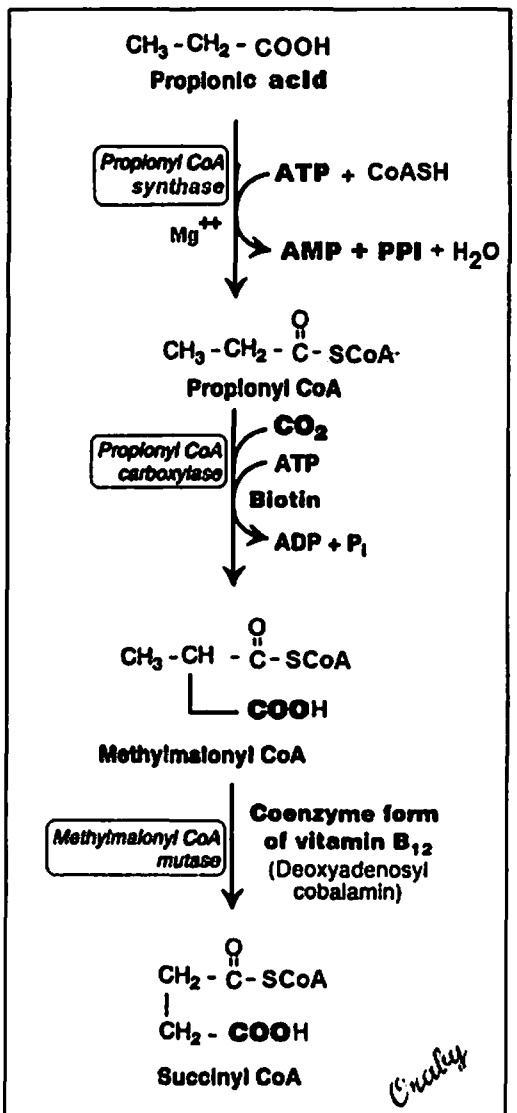
1. Glutamate is converted into α-ketoglutarate by transamination reaction:



2. α -Ketoglutarate is converted into malate as follows :
   α -Ketoglutarate → Succinyl CoA → Fumarate → Malate.
3. Malate then passes out the mitochondria and join common pathway to glucose (as indicated in the diagram).

C. Gluconeogenesis from propionic acid:

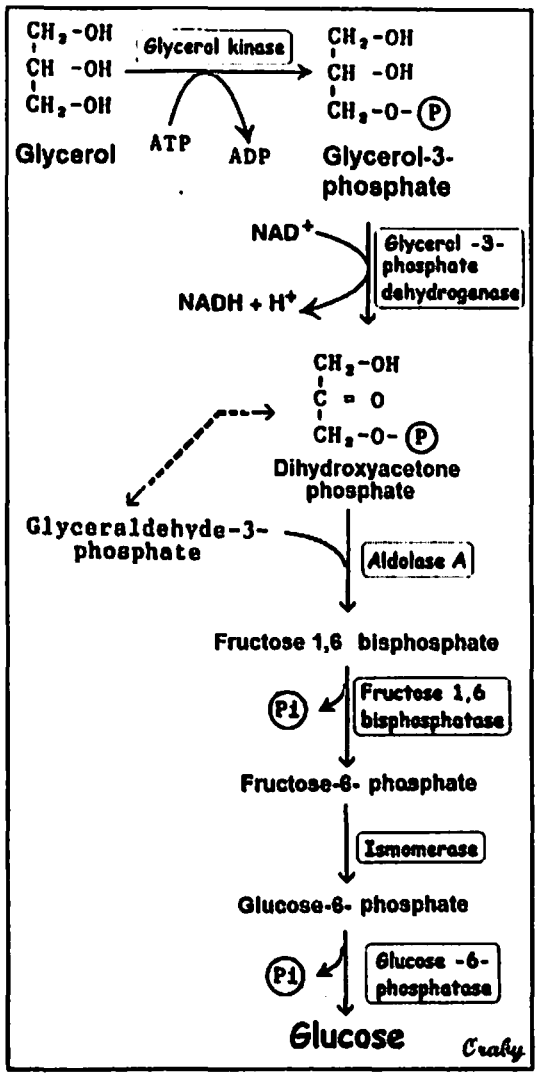
1. This occurs only in **ruminants** and not in human.
2. Propionic acid is converted into succinyl CoA as shown in the figure.
3. Succinyl CoA is then converted into malate:
   Succinyl CoA → Fumarate → Malate.
4. Malate passes out the mitochondria and join common pathway to glucose (as indicated in the diagram).



Gluconeogenesis from propionic acid.

D. Gluconeogenesis from glycerol:

1. Glycerol is mobilized from adipose tissue during fasting. Two molecules of glycerol are used to form glucose:



Note:

Two important cycles are related to gluconeogenesis: Cori cycle (see glycolysis, part II) and alanine glucose cycle (see protein metabolism, part III).

VI. *Energy cost of gluconeogenesis:*

Gluconeogenesis is an endergonic process (anabolic). For conversion of two molecules of pyruvate to one molecule of glucose, 4 molecules of ATP, 2 molecules of GTP and two molecules of NADH, H^+ are utilized as follows:

A. **ATP and GTP:**

Two pyruvate → Two oxaloacetate (-2ATP)

Two Oxaloacetate → Two phosphoenolpyruvate (-2 GTP)

Two 3 phosphoglycerate → Two 1,3 Bisphosphoglycerate (-2 ATP)

B. **$NADH,H^+$:**

Two 1,3 Bisphosphoglycerate → Two glyceraldhyde-3-phosphate (-2 NADH+H^+)

VII. *Regulation of gluconeogenesis:*

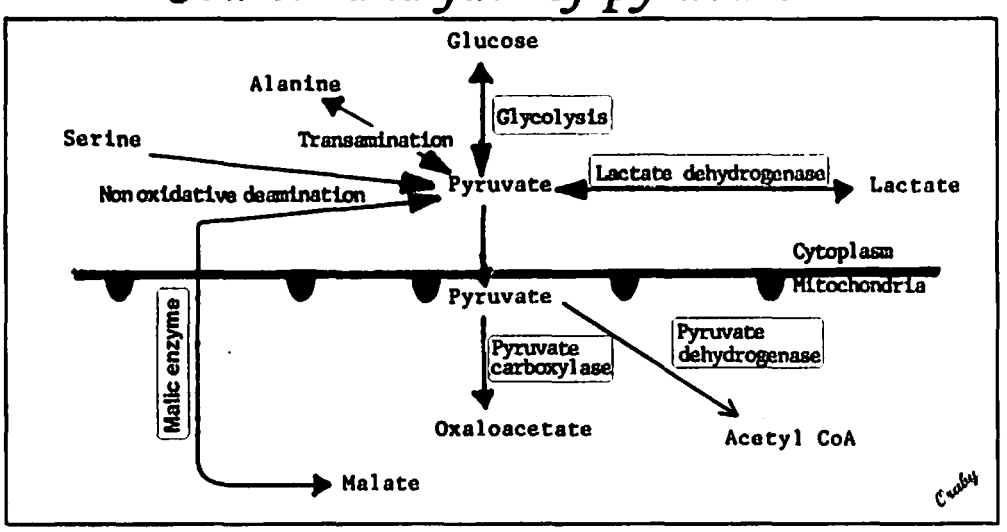
A. Hormonal regulation:

1. **Glucocorticoids e.g. *cortisol*:** stimulate gluconeogenesis by the following mechanisms:
   a) They **Induce: (stimulate)** the synthesis of gluconeogenesis enzymes which are: pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6 bisphosphatase and glucose-6-phosphatase.
   b) Glucocorticoids stimulate protein catabolism by tissues → ↑ glycogenic amino acids available for gluconeogenesis.
2. **Glucagon**: Stimulates gluconeogenesis by lowering the level of fructose 2,6 bisphosphate (see regulation of glycolysis).
3. **Insulin:** Inhibits gluconeogenesis. It acts as repressor (inhibitor) for synthesis of enzymes of gluconeogenesis: pyruvate carboxylase etc.

B. Acetyl CoA and ATP:

1. Stimulate gluconeogenesis by inhibiting glycolysis (through inhibiting phosphofructokinase-1) and stimulate gluconeogenesis (by stimulating fructose 1,6 bisphosphatase).
2. Acetyl CoA also stimulates pyruvate carboxylase (gluconeogenesis) and inhibit pyruvate dehydrogenase (oxidation).

Sources and fate of pyruvate



A. Sources:

1. **Glucose oxidation:** glycolysis.
2. **Lactate:** by lactate dehydrogenase.
3. **Malate:** by malic enzyme.

4. **Alanine:** by transamination.
5. **Serine:** by non-oxidative deamination.
6. **Other amino acids:** methionine, cysteine, threonine and glycine.

B. **Fate:**

1. **Glucose formation:** gluconeogenesis.
2. **Lactate formation:** by lactate dehydrogenase.
3. **Malate formation:** by malic enzyme.
4. **Alanine formation:** by transamination.
5. **Oxaloacetate formation:** by pyruvate carboxylase.

Metabolism of monosaccharides

Galactose metabolism

I. ***Importance of galactose:*** In the form of UDP-galactose:

A. Synthesis of lactose (=milk sugar).

B. Synthesis of glycolipids (cerebrosides).

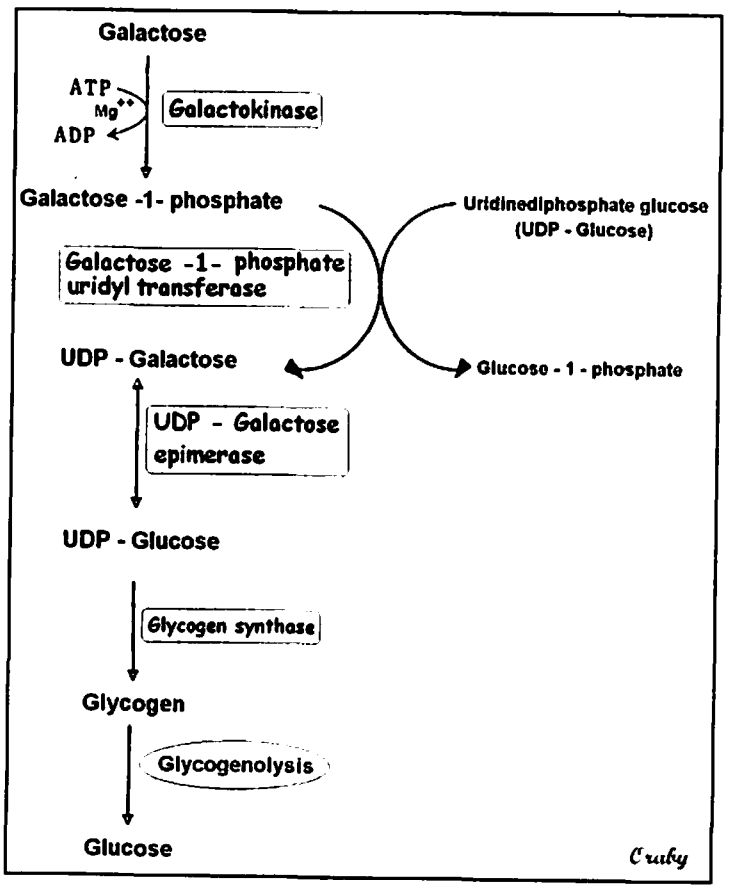
C. Synthesis of glycoproteins and proteoglycans.

D. Synthesis of glycosaminoglycans.

II. ***Conversion of galactose into glucose:***

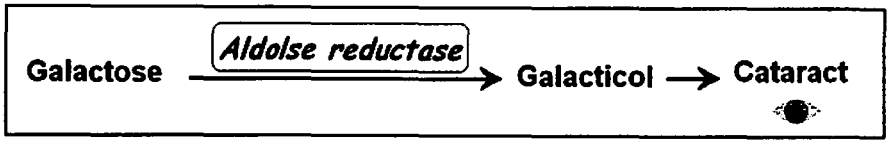
A. **Site:** Liver.

B. **Steps:**



C. **Galactosemia:**

1. **Definition:** It is increase blood galactose concentration due to inability to metabolize galactose.
2. **Causes:** Inherited enzyme deficiency of:
   a) Galactokinase.
   b) Galactose-1-P uridyl transferase.
   c) Epimerase.
3. **Effect:**
   a) **Cataract** (=opacity of eye lens):
   Galactose in the eye is reduced by an enzyme called aldose reductase into galacticol, which accumulates causing cataract.



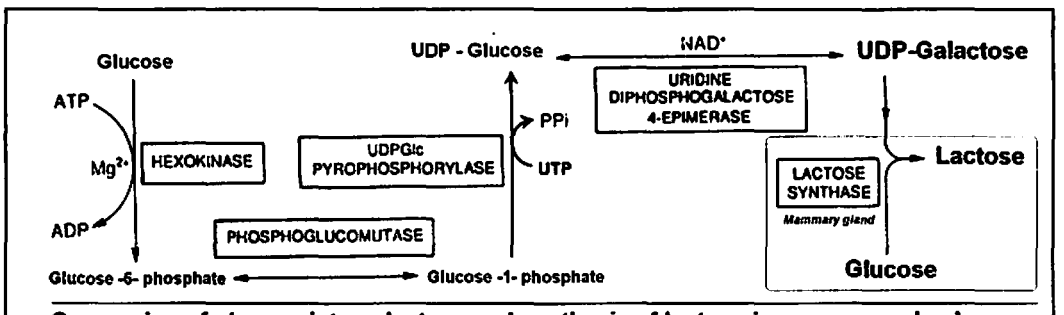
   b) Liver failure.
   c) Mental retardation.
   d) **Galactosuria**: excretion of galactose in urine.

III. *Conversion of glucose into galactose in mammary gland and lactose synthesis:*

Lactose is a disaccharide formed of β -galactose attached to α-glucose by β1-4 bonds. It is called milk sugar.

A. **Steps:**

1. Glucose is first converted into UDP-galactose.



Conversion of glucose into galactose and synthesis of lactose in mammary gland.

2. UPD-Galactose reacts with a molecule of glucose in the presence of lactose synthase enzyme to form lactose.

Fructose metabolism

I. Dietary Sources of fructose:

A. **Sucrose:** (table sugar): Hydrolysis of sucrose ➔ glucose and fructose.

B. **Fructose as a monosaccharide** is present in honey and in many fruits and vegetables.

II. Importance of fructose:

A. **Energy production:** 15% of daily energy is derived from fructose.

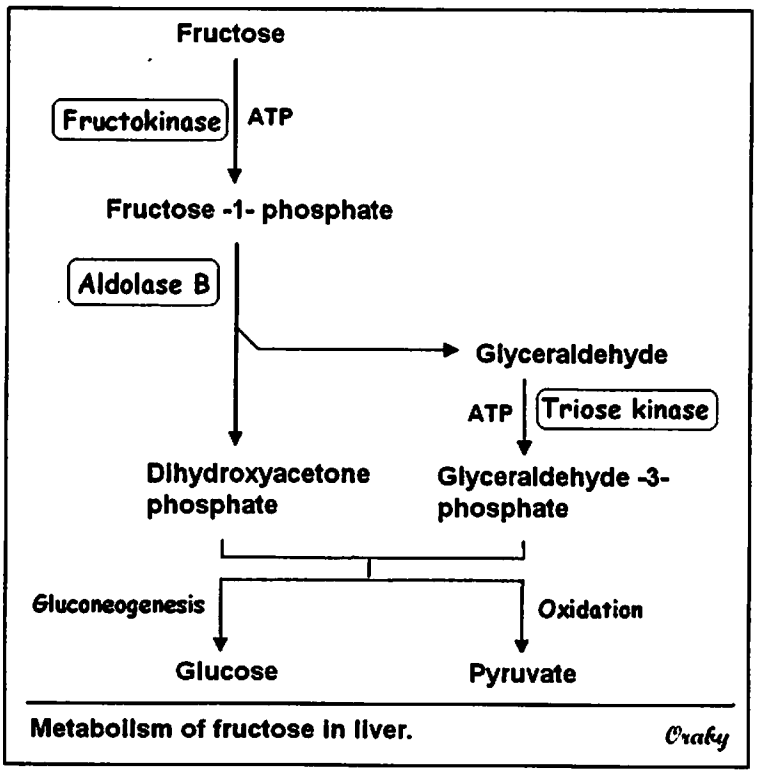
B. **Fructose is the major energy** source for spermatozoa in the seminal vesicle.

> **Note:** Entry of fructose to the tissue cells is not insulin dependent.

III. Metabolism of fructose:

A. **In the liver:**

1. **Liver contains fructokinase enzyme,** which phosphorylates fructose into fructose-1-phosphate.
2. Fructose-1-phosphate **by aldolase B enzyme** ➔ Dihydroxyacetone phosphate + glyceraldehyde.
3. Glyceraldehyde ➔ glyceraldehydes 3- phosphate.

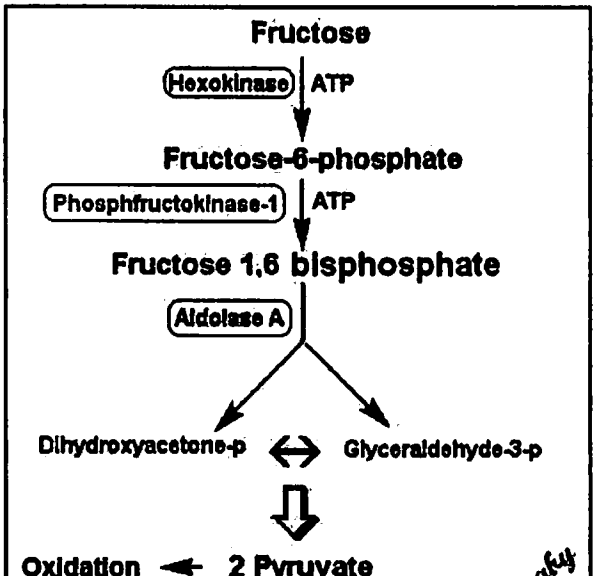


Metabolism of fructose in liver. Oraby

4. Glyceraldehyde-3-p + Dihydroxyacetone p may undergo:
   a) Glucose formation (gluconeogenesis): main pathway.
   b) Oxidation to pyruvate (glycolysis).

B. In extra-hepatic tissues:

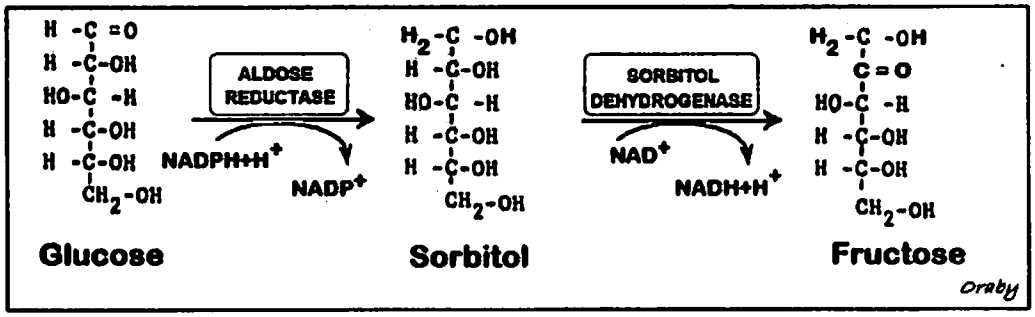
1. Because fructokinase is not available in muscles and adipose tissue, fructose is metabolized by hexokinase and other enzymes into pyruvate ➔ oxidation.



Fructose metabolism in extrahepatic tissues.

C. In the testis (seminal vesicle), lens, peripheral nerves and renal glomeruli:

1. Glucose is converted into fructose through sorbitol formation:



2. Fructose is the main **nutrient for sperms.**
3. Deficiency of fructose in semen correlates with male infertility.

IV. *Genetic disorders of fructose metabolism:*

A. **Essential fructosuria:**

1. **Cause:** Due to deficiency of ***fructokinase*** enzyme.
2. **Effect:** Not serious condition. The excess accumulated fructose is lost in urine (fructosuria).

B. **Hereditary fructose intolerance:**

1. **Cause:** Due to deficiency of ***aldolase-B*** enzyme. This leads to accumulation of fructose-1-phosphate.
2. **Effect:** the accumulation of fructose-1-phosphate leads to:
   a) Damage of liver and kidney tissues → Liver and kidney failure.
   b) Inhibition of phosphorylase enzyme. This leads to inhibition of glycogenolysis and hypoglycemia.

Blood glucose

I. *Plasma glucose levels:*

A. **Fasting level** = 65-110 mg/dl (3.6-6.1 mmol/L)

B. **One hour after carbohydrate meal** = 120-150 mg/dl (6.7-8.3 mmol/L).

C. **Two hours after carbohydrate meal (PP)**: 65-140 mg/dl (3.6-7.8 mmol/L).

Note: For glucose 1 millimole/L (mmol/L) = 18 mg/dl.

II. *Sources of blood glucose:*

A. **Dietary carbohydrate:** absorbed glucose following digestion of carbohydrate e.g. starch.

B. **Liver glycogen:** through glycogenolysis. Liver glycogen can supply body with glucose for about 18 hours of fasting.

C. **Amino acids and other metabolites:** (gluconeogenesis): liver and kidney can convert these substrates into glucose.

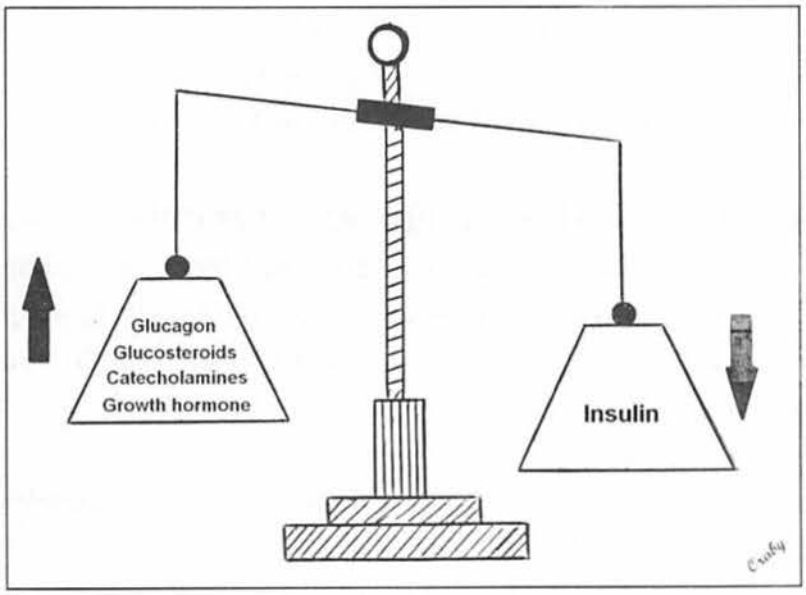
III. *Regulation of blood glucose level:*

A. Blood glucose level is maintained within narrow range 65-150 mg/dl. This is because:

1. Hypoglycemia (low blood glucose level) causes impairment of cerebral function, as brain is very dependent on blood glucose for its energy supply.

2. Hyperglycemia (high blood glucose level) can also cause cerebral dysfunction by its effect on extra cellular osmolality (discussed later).
3. Regulation of blood glucose level can be achieved by hormonal, hepatic and renal mechanisms.

B. Hormonal regulation:



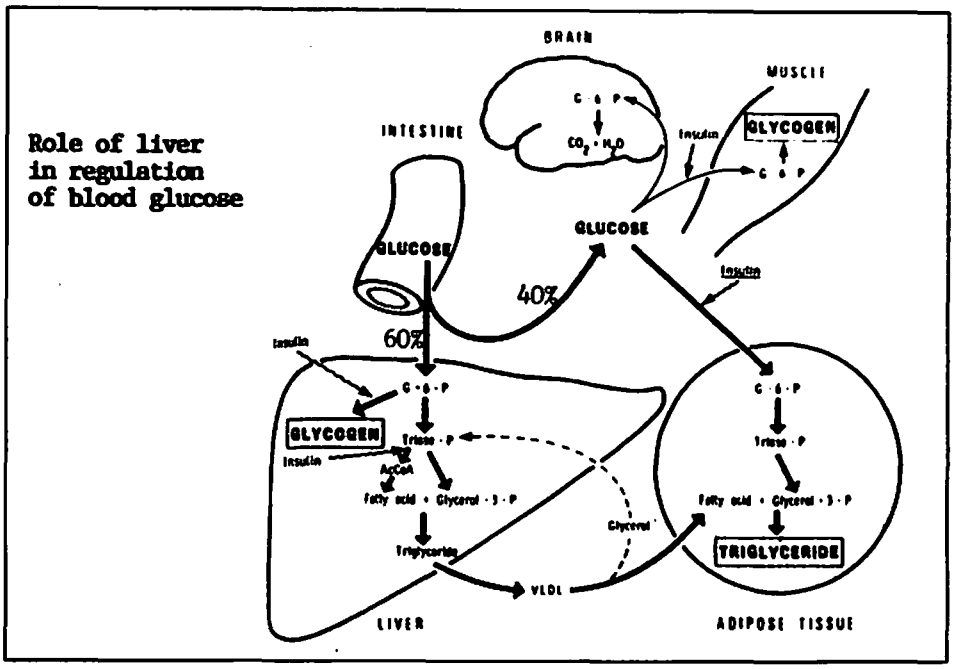
1. **Insulin** is a hormone secreted by beta cells of islets of Langerhans of pancreas (see chapter of hormones). It is the only hormone which reduces blood glucose level through:
   a) **Transfer** of glucose inside the cells.
   b) **Stimulation** of glucose oxidation.
   c) **Stimulation** of glycogen storage (glycogenesis).
   d) **Inhibition** of glycogen breakdown (glycogenolysis).
   e) **Inhibition** of conversion of amino acids and other metabolites into glucose (gluconeogenesis).
   f) **Stimulation** of conversion of glucose into fat (lipogenesis).
2. **Glucagon:** is a hormone that secreted by alpha cells of islets of Langerhans of pancreas. It tends to increase blood glucose level through:
   a) Stimulation of glycogen breakdown (glycogenolysis): by stimulating adenylate cyclase enzyme.
   b) Stimulation of gluconeogenesis.
3. **Catecholamines (epinephrine and norepinephrine):** They are secreted from suprarenal medulla. They tend to increase blood glucose level even if there is hyperglycemia as in

case of stress. They increase blood glucose level through stimulation of glycogenolysis.

4. **Glucocorticoids as cortisol:** They are a group of steroid hormones secreted by suprarenal cortex. They tend to increase blood glucose level through stimulation of gluconeogenesis.
5. **Growth hormone:** It is a hormone secreted from anterior pituitary gland. It antagonizes insulin hormone and tends to increase blood glucose level through:
   a) Inhibition of glucose uptake by cells.
   b) Blocks the insulin action at cell membranes.

C. **Hepatic regulation (role of liver):** Liver plays a very important role in keeping blood glucose level within range. Simply during fasting, liver adds glucose to the blood (glycogenolysis). After carbohydrate meal, it takes up about 60% of the glucose load (glycogenesis).

1. **During fasting:**
   a) Liver adds glucose to blood by glycogenolysis and gluconeogenesis.
   b) It can convert fatty acids (acetyl CoA) released from adipose tissue to ketone bodies, which can be used by other tissues, including brain when glucose is in short supply.
2. **After carbohydrate meal:** Glucose is transported in portal blood to the liver.
   a) About **40%** passes to the blood stimulating insulin secretion and taken up by brain, muscles, adipose tissue and liver.
   b) The remaining **60%** of glucose is taken up by liver, and converted into glucose-6-phosphate, by **glucokinase enzyme:**
      1) This enzyme has **low affinity** for glucose relative to hexokinase found in most tissues.
      2) Glucokinase is stimulated **(induced) by insulin** secreted in response to hyperglycemia. For this reason, less glucose is taken up by liver during fasting.
      3) Glucose-6-phosphate will undergo one of the following **fate in the liver:**
         - Conversion to glycogen (glycogenesis) which is stimulated by insulin.
         - Conversion to fatty acids (lipogenesis). It is also stimulated by insulin.



D. **Renal regulation**: (role of kidney):

1. Circulating blood glucose is filtered in glomerular filtrate and reabsorbed again by certain tubular enzymes. This saves blood glucose from loss in urine .
2. If blood glucose level exceeds certain limits (average 180 mg/dl), glucose will increase in glomerular filtrate and exceeds the capacity of tubular enzymes to reabsorb it. Thus glucose appears in urine (glycosuria). This limit is called renal threshold for glucose reabsorption.

Definition of renal threshold for glucose reabsorption: It is the blood glucose level above which glucose appears in urine. (Average **180 mg/dl**).

3. **Low renal threshold:** Where glucose appears in urine even when blood glucose level is low (may be just 100 mg/dl). **This occurs in:**
   a) Normally in some persons due to defective tubular enzymes for glucose reabsorption (diabetes innocence).
   b) In 20% of pregnant females.
4. **High renal Threshold:** Where glucose appears in urine in concentration above the average "renal threshold" (which may reach 220 mg/dl or more). This occurs in:
   a) Elderly people due to reduced glomerular filtration rate.
   b) In cases of diabetes associated with renal damage.

Variations in blood glucose

Hypoglycemia

I. Definition:

A. It is the decrease of blood glucose concentration below normal fasting average concentration: **less than 65 mg/dl. Symptoms** of hypoglycemia appear if blood glucose concentration becomes less than**45 mg/dl.**

II. Effect of hypoglycemia:

A. Hypoglycemia causes cerebral dysfunction (as brain and nervous tissue are dependent on glucose as a source of energy).

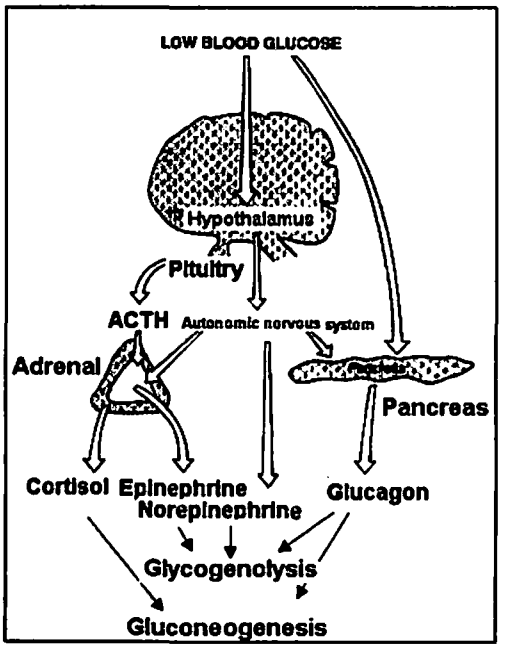
B. If hypoglycemia is severe and prolonged, it causes coma (hypoglycemic coma) and then death.

III. Mechanism that corrects hypoglycemia: Hypoglycemia activates:

A. **α Cells of islets of Langerhans** ➔ Glucagon ➔ Glycogenolysis ➔ ↑ Blood glucose.

B. **Receptors in hypothalamus:** This stimulates:

1. Secretion of **epinephrine** (mediated by autonomic nervous system) ➔ ↑ Glycogenolysis ➔ ↑ Blood glucose.
2. Secretion of **anterior pituitary** hormones:
   a) **Growth hormone:** insulin antagonist.
   b) **ACTH** ➔ Stimulation of suprarenal cortex ➔ Glucocorticoids ➔ ↑ Gluconeogenesis ➔ ↑ Blood glucose.

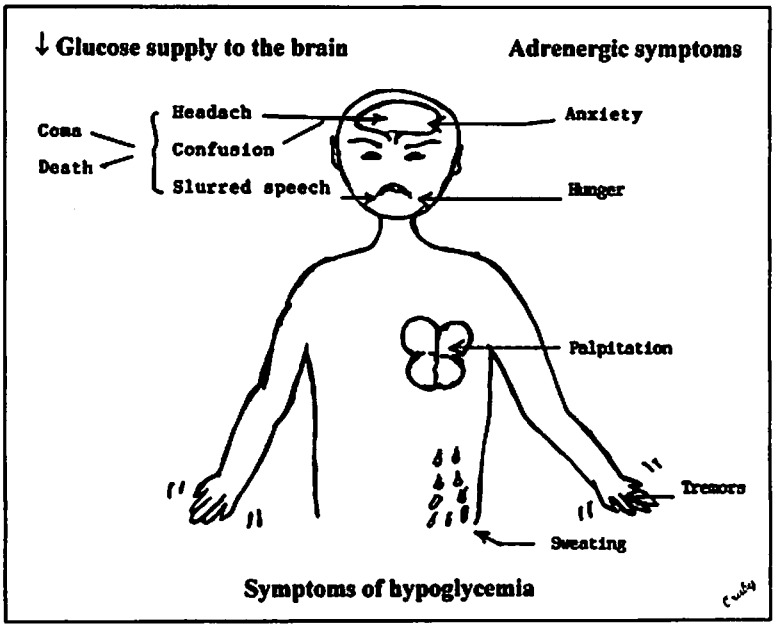


Note:

1) **Glucagon and epinephrine are most important in the acute, short-term regulation of blood glucose levels.**

2) **Glucocorticoids and growth hormone play a role in long-term regulation of blood glucose levels.**

IV. *Symptoms of hypoglycemia:*



A. Adrenergic symptoms (that is, symptoms mediated by elevated epinephrine) ➔ *Anxiety, palpitation, tremors and sweating and hunger.*

B. Symptoms due to impaired glucose supply to the brain ➔ Impairment of brain function ➔ *Headache, confusion, slurred speech, coma and death.*

V. *Types of hypoglycemia:* It may be divided into 2 types, fasting and stimulative.

A. Fasting hypoglycemia:

1. It is the inability to maintain normal glucose concentration in the fasting state.
2. It is usually due to organ diseases.
3. **Causes:**
   a) **Pancreatic disease:** disorders affecting β-cells of islets of Langerhans e.g. ***insulinoma*** (=Pancreatic β cell tumor) ➔ ↑↑ insulin secretion.
   b) **Liver diseases:** leads to ↓ glycogenolysis and ↓ gluconeogenesis:
      1) Hepatocellular damage e.g. hepatic carcinoma
      2) Glycogen storage diseases.
      3) Prolonged starvation: due to depletion of liver glycogen.

c) **Adrenocortical diseases:** ➔ lead to decreased secretion of epinephrine (↓ glycogenolysis) and glucocorticoids (↓ gluconeogenesis).

B. Stimulative (reactive) hypoglycemia:

1. It occurs due to some stimuli usually after taking a meal.
2. **Causes:**

a) **Drugs and poisons:**

1) **Therapeutic insulin administration:** over dosage of insulin during treatment of diabetes can lead to hypoglycemia.
2) **Over dosage of sulphonylurea:** it is an oral hypoglycemic drug that stimulates β-cells to produce insulin.
3) **Liver poisons:** as chloroform and phosphorus.
4) **Alcohol (ethanol) ingestion:**
   - Excessive alcohol ingestion is a common cause of hypoglycemia.
   - Metabolism of alcohol ➔ ↑ $NADH+H^+$ levels in the liver ➔ Inhibits gluconeogenesis ➔ Hypoglycemia.

b) **Postgastrectomy:** i.e. after partial removal of stomach. Glucose is absorbed rapidly which leads to stimulation of excessive insulin secretion.

c) **Leucine hypersensitivity of infancy and childhood:** Leucine amino acid in the diet stimulates β-cells to secrete insulin. In sensitive infants, there is excessive insulin release.

d) **Inborn errors of metabolism:** as in:

1) **Galactosemia:** due to deficiency of galactose-1-phosphate uridyl transferase.
2) **Hereditary fructose intolerance:** due to deficiency of aldolase B enzyme ➔ Inhibition of phosphorylase ➔ Inhibition of glycogenolysis.

Types and causes of hypoglycemia:

Types	Organ or cause	Examples
Fasting hypoglycemia	**Pancreas**	Insulinoma.
	Other endocrine glands	Adrenocortical hypofunction.
	Liver	Prolonged starvation. Hepatocellular damage. Glycogen storage diseases.
Stimulative Hypoglycemia	**Drugs and poisons**	↑dose of insulin or ↑dose of sulphonylurea. Liver poisons: chloroform, Alcohol (ethanol).
	Postgastrectomy **Leucine hypersensitivity**	
	Inborn errors of metabolism	• Galactosemia • Hereditary fructose intolerance.

Hyperglycemia

I. ***Definition:***

It is the rise of blood glucose above normal average level.

II. ***Causes:***

1. **Diabetes mellitus**: *Most common cause* (discussed later).
2. In patients receiving intravenous fluid containing glucose.
3. Temporarily in severe stress.
4. After cerebro-vascular accidents.
5. Disturbance in hyperglycaemic hormones.

Diabetes mellitus

I. ***Definition:***

A. It is an endocrinc disease caused by a relative or absolute **deficiency of insulin** hormone.

B. It is characterized by a **chronic hyperglycemia.**

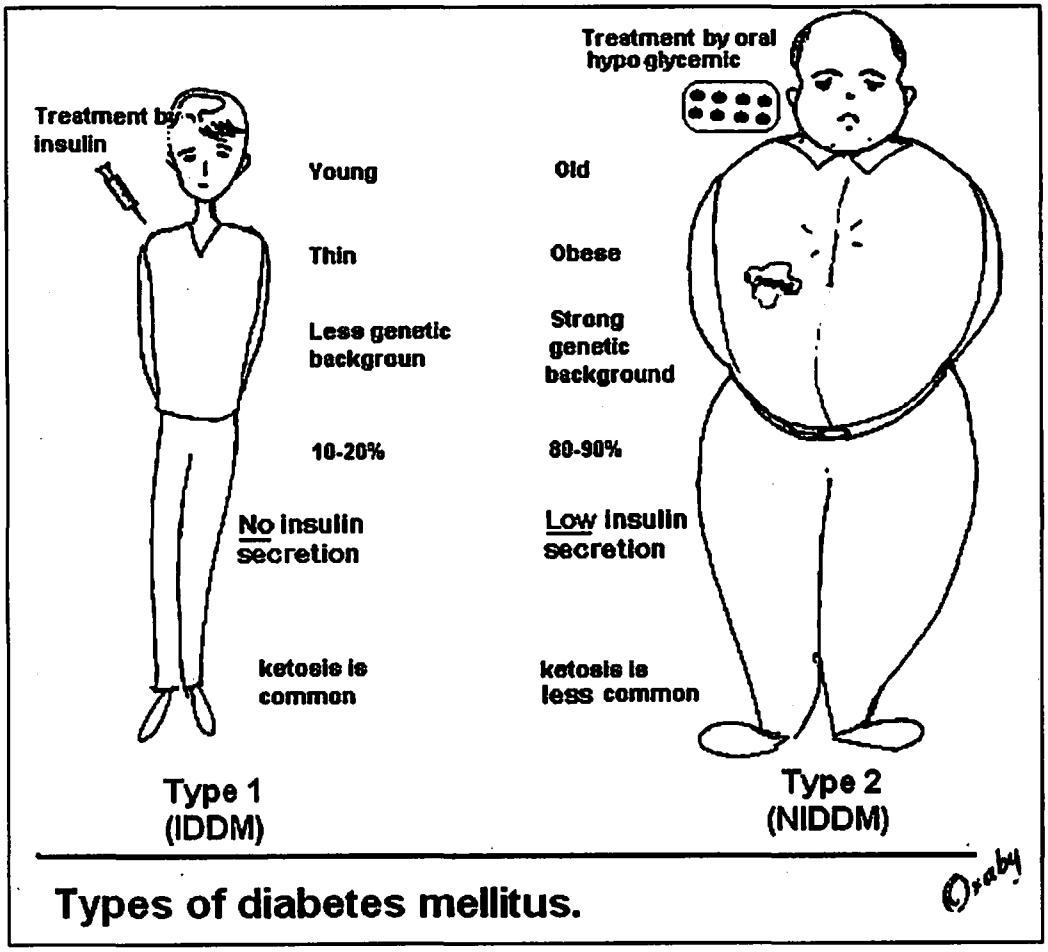
C. **Glycosuria** (presence of glucose in urine) is usually present.

Note: The word DIABETES means increase urine volume.

II. ***Types of diabetes:*** 2 Types; insulin dependent and non-insulin dependent diabetes mellitus.

Comparison of two types of diabetes mellitus

	Type 1 Insulin-dependent diabetes mellitus (IDDM)	Type 2 Non-insulin-dependent diabetes mellitus (NIDDM)
Synonym (other names)	juvenile-onset diabetes	adult-onset diabetes
Age of onset	Usually during childhood or puberty **(young)**	Frequently after age 35 **(old).**
Nutritional status at time of onset of disease	Frequently undernourished **(thin)**	Obesity usually present
Prevalence	10-20% of diagnosed diabetics	80-90% of diagnosed diabetics
Genetic predisposition	Moderate	Very strong
Defect or deficiency	β-cells destruction **(no insulin).**	Inability of β-cells to produce appropriate quantities of insulin or insulin resistance.
Ketosis	Common	Rare
Plasma insulin	Low to absent	Normal to high
Acute complications	Ketoacidosis	Hyperosmolar coma
Oral hypoglycemic drugs	No response	Responsive
Treatment with insulin	Always necessary	usually not required

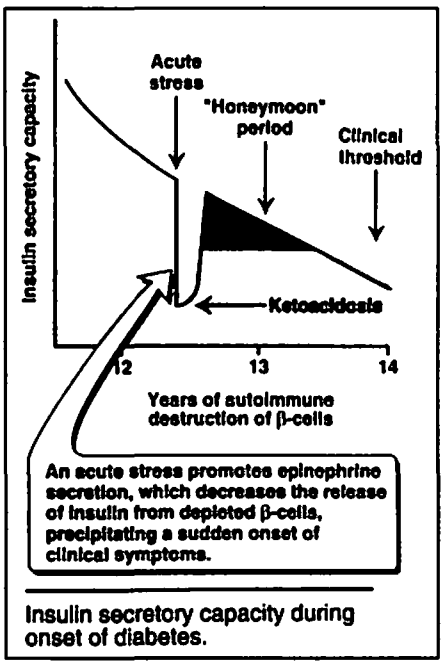


Types of diabetes mellitus.

A. **Insulin-dependent (type I) diabetes mellitus (IDDM):** This type is less common (10-20%) and characterized by an **absolute deficiency** of insulin caused by:

1) **Destruction of β-cells** of the pancreas.
2) This destruction may be due to **viral infection** or formation of **antibodies against β cells.**

- Patients of this type are usually
  - **young** and
  - **develop ketosis.**



Insulin secretory capacity during onset of diabetes.

B. **Non-Insulin dependent (type II) diabetes mellitus (NIDDM):** This type is the most common form of the disease (80-90%) and characterized by a relative deficiency of insulin caused by:

1) **Dysfunction of β-cells** of the pancreas.
2) **Resistance to insulin** action at cellular level. This may explain why some patients of NIDDM show normal or even elevated plasma insulin.

- Patients of this type are usually
  - **Old** and
  - **Obese** at the onset of diabetes.
  - Strong **genetic** background

III. ***Biochemical disturbance of diabetes mellitus:*** insulin deficiency causes disturbance in carbohydrate, lipids, protein, vitamins and minerals metabolism.

A. **Carbohydrate metabolism:** insulin deficiency leads to decrease glucose uptake by tissues, ↓ glucose oxidation, ↑ gluconeogenesis and ↑ Glycogenolysis. This leads to:

1. ↓ **Intracellular glucose** ➔ hunger pain ➔ **Polyphagia (excessive eating).**
2. ↑ **Blood glucose (hyperglycemia),** this causes ↑ **Plasma osmolality** ➔ dehydration:
   a) Dehydration of brain cells ➔ Hyperglycemic, hyperosmolal coma.

b) Dehydration of body cells → Sense of thirst ➔ Excessive drinking **(polydepsia).**

c) Symptoms and sign of dehydration.

3. **Glycosuria:** If blood glucose level exceeds renal threshold ➔ Glucose appears in urine **(glycosuria).** This will leads to ↑ osmotic diuresis that causes:
   a) Excessive and frequent urination **(polyurea).**
   b) Loss of water soluble vitamins e.g. B_1.
   c) Loss of minerals e.g. Na^+ and K^+.

B. **Protein metabolism:** insulin deficiency leads to increased protein breakdown and stimulation of gluconeogenesis. i.e. conversion of amino acids to glucose. This results in :

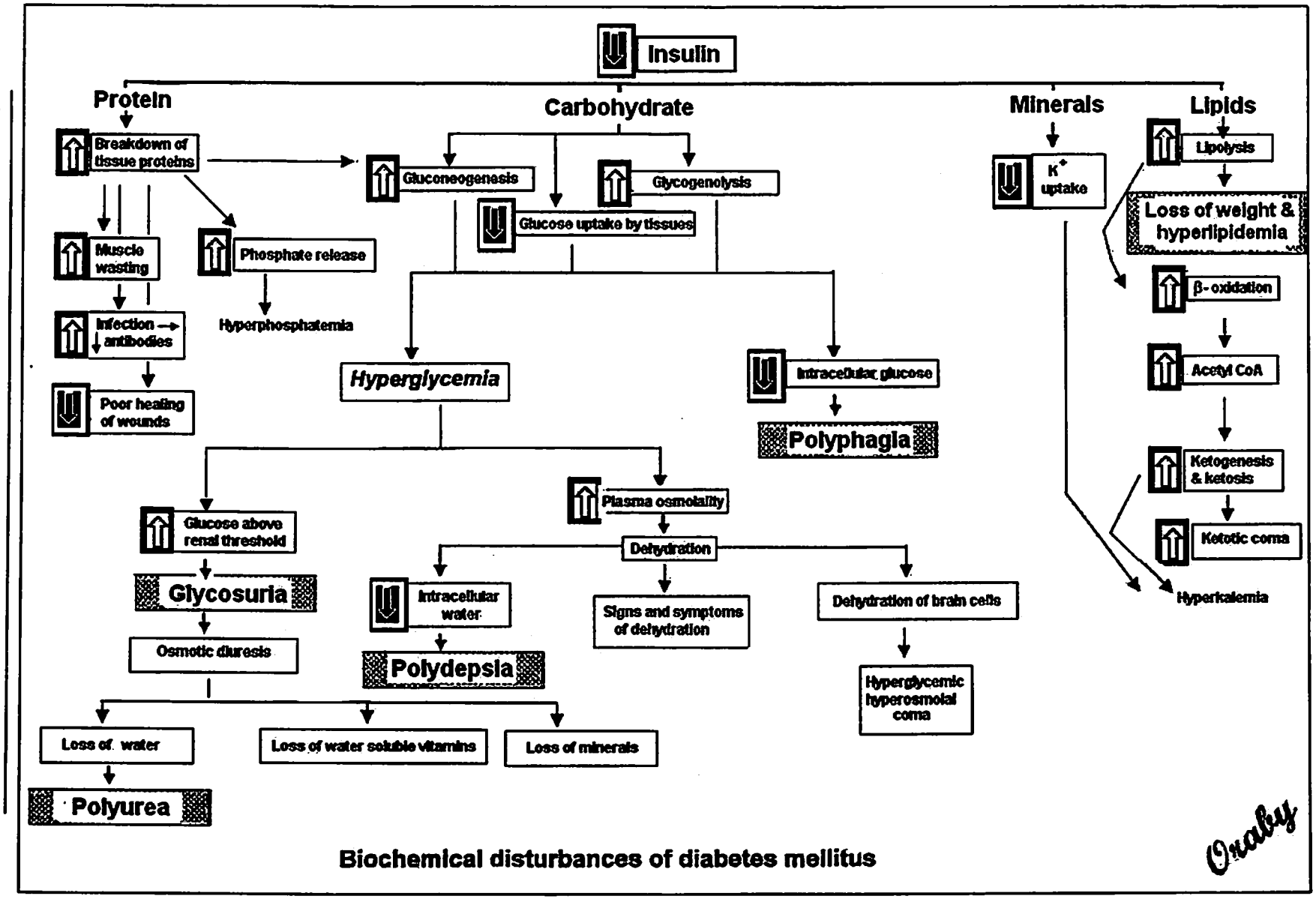
1. Phosphate release ➔ **hyperphosphatemia.**
2. Excessive breakdown of tissue proteins causing **muscle wasting.**
3. Decreased **antibody formation** causing low resistance and infection.
4. **Poor healing of wounds.**

C. **Lipid metabolism:** Insulin deficiency leads to excessive lipolysis in adipose tissue ➔ mobilization of free fatty acids and glycerol to the blood ➔ Then to the liver and other tissues. This leads to:

1. Loss of weight.
2. Hyperlipidemia ➔ Atherosclerosis.
3. Fatty liver (Re-estrification of fatty acids again with glycerol to form triacylglycerol in liver).
4. Excessive ketone bodies formation ➔ Ketonemia ➔ Ketosis. This leads to:
   a) Coma: ketotic coma.
   b) Hyperkalemia.

D. **Microangiopathy:**

1. It is a degeneration that affects small blood vessels as capillaries especially those of the kidneys and retina of the eyes.
2. Thus two of common **chronic complications** of diabetes mellitus are **renal failure** and **blindness.**


Insulin
Protein
Carbohydrate
Minerals
Lipids
Breakdown of tissue proteins
Gluconeogenesis
Glycogenolysis
Glucose uptake by tissues
K+ uptake
Lipolysis
Loss of weight & hyperlipidemia
Muscle wasting
Phosphate release
Hyperphosphatemia
Infection → ↓ antibodies
Poor healing of wounds
Hyperglycemia
Intracellular glucose
Polyphagia
β- oxidation
Acetyl CoA
Ketogenesis & ketosis
Ketotic coma
Hyperkalemia
Glucose above renal threshold
Glycosuria
Osmotic diuresis
Plasma osmolality
Dehydration
Intracellular water
Polydepsia
Signs and symptoms of dehydration
Dehydration of brain cells
Hyperglycemic hyperosmolal coma
Loss of water
Loss of water soluble vitamins
Loss of minerals
Polyurea
Biochemical disturbances of diabetes mellitus
Orabey

IV. *Diagnosis of diabetes mellitus:*

Symptoms:
1- Fasting plasma glucose.
2- Postprandial plasma glucose.
No symptoms:
1- OGTT

A. **If a patient has symptoms** of diabetes mellitus, the following tests may be done:

1. ***Measurement of fasting plasma glucose level*** (8-12 hours after last meal):
   a) If concentration is 65-110 mg/dl → **Normal blood glucose.**
   b) If concentration is 110-126 mg/dl → **Impaired glucose utilization.**
   c) If concentration is > 126 mg/dl → **Diabetes mellitus.**
2. ***Measurement of two-hours postprandial plasma glucose level:*** (2 hours after last meal):
   a) If concentration is 65-140 mg/dl → **Normal blood glucose.**
   b) If concentration is 140-200 mg/dl→**Impaired glucose utilization.**
   c) If concentration is > 200 mg/dl → **Diabetes mellitus.**

	Glucose concentration (mg/dl)	
	Fasting	**Two hours after last meal**
Normal	65-110 mg/dl	65-140 mg/dl
Impaired glucose tolerance	110-126 mg/dl	140-200 mg/dl
Diabetes mellitus	More than 126 mg/dl	More than 200 mg/dl

Summary of diagnostic criteria for patients with symptoms of diabetes mellitus (WHO criteria).

B. **If patient has no symptoms:** ***Oral glucose tolerance test (OGTT) is done:***

1. **Indication of the test:** There are only two indications for performing OGTT:
   a) People with no or mild symptoms of diabetes mellitus.
   b) To determine the renal threshold for glucose.
2. **Procedure:**
   a) Fasting plasma sample is taken after overnight fast (8-12 hours).
   b) Then patient is given 75 grams glucose orally.
   c) Plasma and urine glucose concentrations are determined in fasting and at 30 minutes intervals for the next 3 hours after glucose ingestion. Then draw a curve.
3. **Explanation of normal glucose tolerance curve:**
   a) **Fasting level:** 65-110 mg/dl.

b) The plasma glucose level rises and reaches the maximum in one hour (120-150 mg/dl). Normally the maximum value equals 1.5 fasting value. ***The ascending limb of the curve represents glucose absorption.***

Thus any cause impairing glucose absorption as diarrhea will make the glucose level reaches the maximum in much slower rate (more than one hour).

c) **The plasma glucose returns to fasting level after 2 hours:**

1) ***The descending limb represents glucose utilization by the tissues in response to insulin secretion.*** In diabetes mellitus, glucose returns to fasting level in much slower rate (more than 2 hours).
2) Insulin secretion continues till the plasma glucose level becomes less than normal fasting level. Then insulin secretion stops and plasma glucose level returns once more to normal fasting level. This is called **hypoglycemic response or insulin overshoot.** The return to normal level is due to secretion of glucagon and epinephrine in response to low plasma glucose level.

d) Normally, **all urine samples contain no glucose** as all plasma glucose levels are below the normal threshold.

4. **WHO criteria for diagnosis of diabetes mellitus using oral glucose tolerance test :**

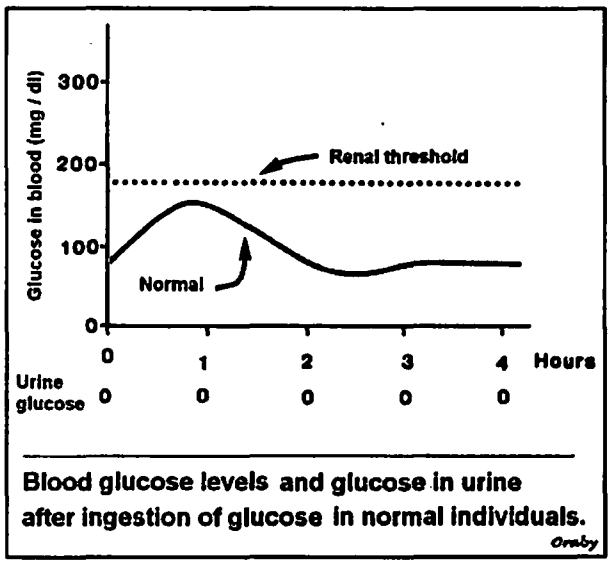
a) fasting plasma glucose is greater than 126 mg/dl (7 mmol/L) .
b) At least, one of the intermediate (30, 60, 90 min) plasma specimens has plasma glucose greater than 200 mg/dl.
c) At any urine specimen, if the rate of glomerular filtration of glucose exceeds that of tubular reabsorption in the kidney (renal threshold), glucose will appear in urine.

5. **Glucose tolerance curve of renal glycosuria:**

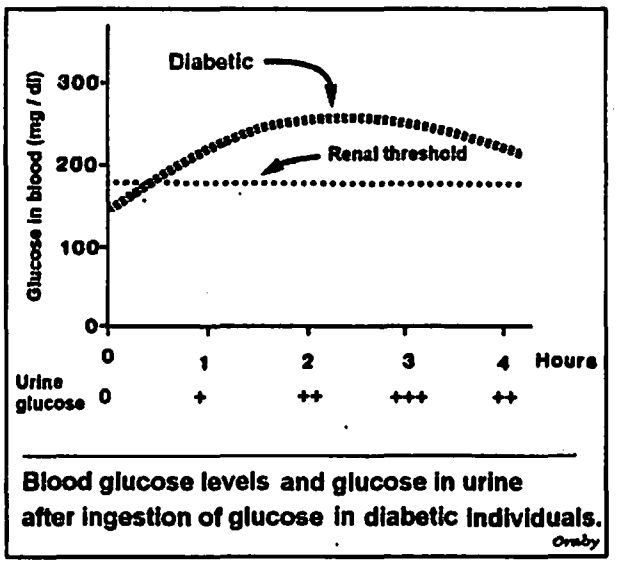
a) The curve is normal, indicating normal glucose utilization and no insulin deficiency.

b) Abnormalities lie in appearance of glucose in some or all urine specimen.

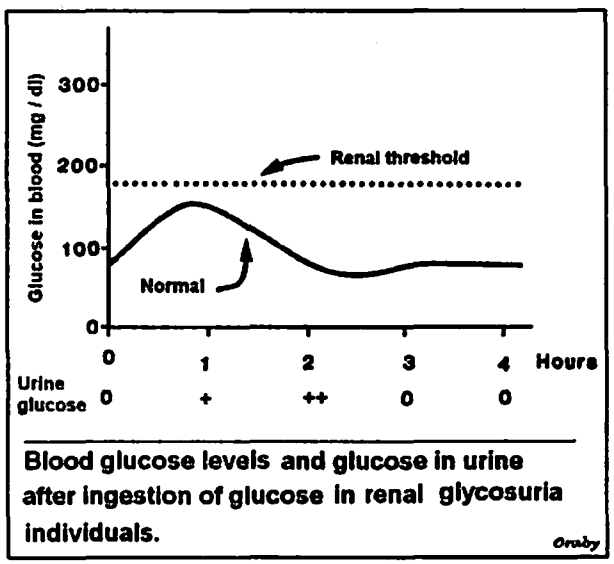
6. **Hypoglycemic glucose tolerance curve:** Fasting level is below normal, maximum rise is below normal and returns to fasting level are very rapid.



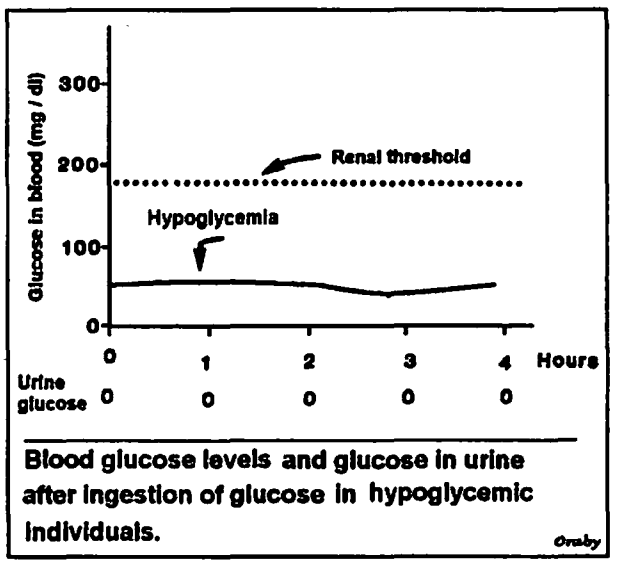
Blood glucose levels and glucose in urine after ingestion of glucose in normal individuals.



Blood glucose levels and glucose in urine after ingestion of glucose in diabetic individuals.



Blood glucose levels and glucose in urine after ingestion of glucose in renal glycosuria individuals.



Blood glucose levels and glucose in urine after ingestion of glucose in hypoglycemic individuals.

C. Investigations for follow up of diabetes mellitus:

1. Glucose in urine (glycosuria):

a) Glucose appears in urine if blood glucose level exceeds renal threshold (180 mg/dl).

b) Glycosuria ***is not used for diagnosis of diabetes mellitus***, as there are other causes of glycosuria.

c) Glycosuria is used rather for follow up (monitoring) of effectiveness of treatment of diabetes.

2. Glycated proteins: all proteins including hemoglobin and albumin undergo slow, non-enzymatic, covalent formation of glycated protein (protein + glucose). The rate of formation of such glycoprotein is related to the concentration of glucose over the life span of that protein.

a) **Glycated hemoglobin:** (formerly named glycosylated hemoglobin):

1) Hemoglobin A is the major hemoglobin in adults(97%) .

2) Normally, about 5-8% of hemoglobin A reacts non-enzymatically with glucose to form a derivative known as **glycated hemoglobin** or HBA_{1c}. This glycated hemoglobin remains over the life span of RBCs (120 days).

3) The concentration of glycated hemoglobin is directly proportional to the glucose level over the life span of RBCs.

4) In diabetes mellitus with uncontrolled hyperglycemia, glycated hemoglobin will be higher than normal (may reach 12% or more of the total hemoglobin A).

5) **Importance of the test:** This test is used as an index of diabetic control over 2-3 months. It correlates with the mean plasma glucose concentration during this period. The higher the percentage, the poorer the mean diabetic control.

b) **Glycated albumin (fructosamine):**

1) Plasma albumin has shorter life span (about 3 weeks) than hemoglobin (2 months).

2) Albumin is also glycated non-enzymatically and can be measured as fructosamine.

3) **Importance of the test:** This test is used as an index of diabetic control over 2-3 weeks (life span of albumin).

This is very useful in short-term evaluation of glucose control (2 weeks) as in pregnancy.

3. Microalbuminuria:

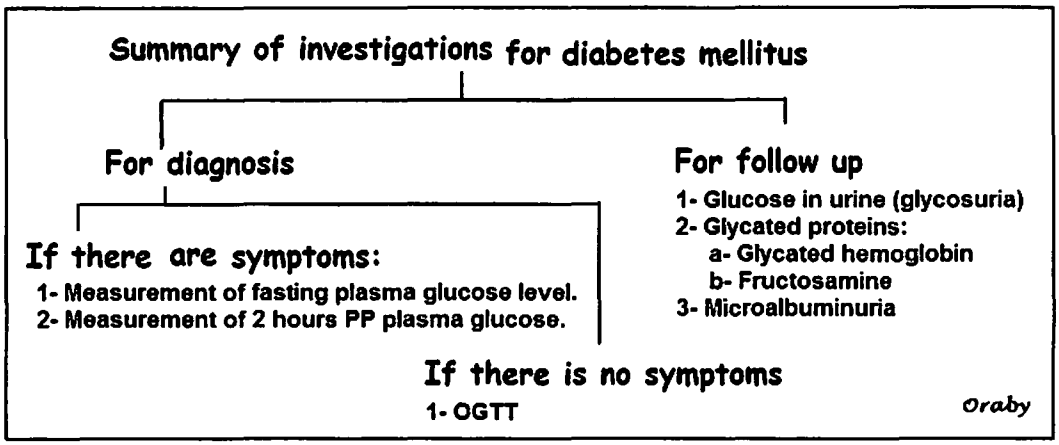
a) **Normally**, proteins (mostly albumin) are excreted in urine in trace amounts (less than 20-30 mg/L).

b) Albuminuria means excretion of protein in amount more than 200 mg/L.

c) Excretion of very small amount of protein or albumin (30-200 mg/L) is called microalbumnuria.

1) It indicates early affection of kidney as in diabetes mellitus.

2) It cannot be detected by ordinary methods (as heat coagulation test) and needs special techniques for its detection.



V. ***Coma in diabetes mellitus:*** Three types of coma may result in advanced uncontrolled diabetes. These are:

A. **Ketotic coma:** due to acidosis (ketosis).

B. **Hyperglycemic, hyperosmolar, nonketotic coma:** due to hyperglycemia, increased osmolality and dehydration of brain cells. Here, there is neither ketosis nor fall in blood pH.

C. **Lactic acidosis:** due to hyperlactatemia. This type may occur in some patients who receive an oral hypoglycemic drug called: Phenformin (cidophage).

Note: Insulin increases the transfer of potassium and inorganic phosphate into cells. In case of treatment of diabetic coma with intravenous insulin and glucose, it may be fatal if potassium is not given at the same time due to hypokalemia ➔ Cardiac affection.

V. *Types of diabetes:*

A. **Diabetes mellitus:** caused by defective insulin action.

B. **Diabetes insipidus:** hereditary disease caused by defective action of antidiuretic hormone.

C. **Diabetes innocence (renal diabetes):** caused by low renal threshold for glucose reabsorption.

D. **Bronze diabetes:** It is a hereditary disease caused by excessive absorption of iron and its precipitation in tissues as :

1. **Skin:** causing its bronze discoloration.
2. **Pancreas:** causing diabetes mellitus.
3. **Liver:** causing hepatic cirrhosis.

VII. *Glycosuria:*

A. **Definition:** It is the presence of glucose in urine in amount detectable by ordinary methods.

B. **Causes (types):**

1. ***Diabetes mellitus:*** It is the most common cause of glycosuria. It accounts about 90% of all cases of glycosuria.
2. **Excess excretion of diabetogenic hormones:** as growth hormone, glucocorticoids, epinephrine and glucagon.
3. **Diabetes innocence (renal glycosuria):** due to abnormal low renal threshold for glucose absorption. It may be inherited as an autosomal dominate trait.
4. **Pregnancy glycosuria:**
   a) Occurs in 20% of all pregnancies.
   b) It is due to increase of glomerular filtration rate by about 50% during pregnancy.

C. **Detection of glycosuria:**

1. **Glucose oxidase method:**
   It is a specific test, because the enzyme does not react except with glucose. This is done by urine strips containing this enzyme.
2. **Benedict's test:**
   Benedict's reagent gives colored precipitate with urine contains glucose. It is non-specific semi-quantitative test because it gives positive results with other substances than glucose as vitamin C.

Summary of Carbohydrate pathways

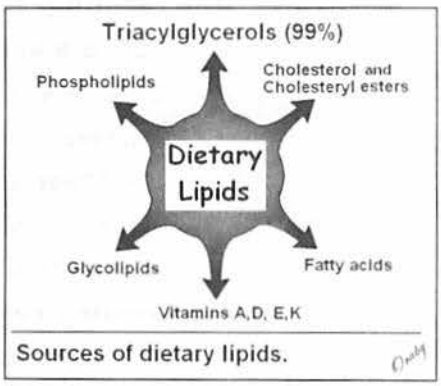
	Glycolysis	Pyruvate to Acetyl CoA	Krebs' cycle	Pentose PP	Uronic acid pathway	Glycogenesis	Glycogenolysis	Gluconeo-genesis
Definition	Glucose to pyruvate or lactate	Pyruvate to Acetyl CoA	Acetyl CoA to H_2O, CO_2 and Energy	is a process that generates NADPH and pentoses	is a process that generates glucuronic acid	Synthesis of glycogen	Breakdown of glycogen	Formation of glucose from non carbohydrate sources
Location	Cytosol	Mitochondria	Mitochondria	Cytosol Liver, RBCs ...	Cytosol Liver	Cytosol Liver& muscle	Cytosol Liver& muscle	Cytosol & mito Liver& kidney
Steps	See	See	See	See	See	See	See	See
Energy	No O_2: 2 ATP O_2: 6 or 8 ATP	3 ATP	12 ATP	NO energy	NO energy	NO energy	NO energy	Consume energy
Functions	Energy DAHP →Fat 2,3 BPG 3 p glyc→serine Pyruv →alanine	Energy	• Energy • Catabolic functions • Anabolic Functions	Production of: • Pentoses • NADPH+H⁺	Production of: Glucuronic acid	Maintains blood glucose concentration in <u>short</u> term fasting		Maintains blood glucose concentration in <u>long</u> term fasting
Key enzymes	Glucokinase Hexokinase PFK PK	Pyruvate dehydrogenase	Citrate synthase Isocitrate dehydrogenase α ketoglutarate dehydrogenase	Gluocose -6-phosphate dehydrogenase	UDP glucose dehydrogenase	Glycogen synthase	Phosphorylase	G-6-phosphatase F 1,6 bisphosphatase PC PEPK Kinase
Regulation	Hormonal Energy Substrate	Phosphorylation dephosphoryla-tion	ATP, NADH+H⁺ Acetyl CoA Long acyl CoA	$NADP^+$, insulin	-	Phosphorylation dephosphoryla-tion		Glucocorticoids Insulin Glucagon
Diseases	PK – HA HK –HA Lactic acidosis	-	-	Favism	Essential pentosuria	Von Gierk's disease		-

DAHP= Dihydroxy acetone phosphate – 2,3 BPG = 2,3 Bisphosphoglycerate – 3 p gly = 3 phosphoglycerate – PFK = Phosphofructokinase – PK = Pyruvate kinase – HA= Hemolytic anemia – HK= hexokinase – PC= Pyruvate carboxylase, PEPK kinase= Phosphoenol pyruvate carboxy kinase

Chapter 3 *Lipids Metabolism*

I. *INTRODUCTION:*

On average, an adult human eats about 100-150 grams lipids per day. The main lipids in diet are triacylglycerols, TG (99%). Diet also contains some phospholipids, cholesterol and fat soluble vitamins.



Sources of dietary lipids.

II. *IMPORTANCE OF LIPIDS IN DIET:*

A. Lipids are one of the main sources of ***energy*** in the body.

B. Lipids supply the body with ***essential fatty acids.***

C. Lipids supply the body with ***fat soluble vitamins.***

D. Lipids make diet ***palatable***.

III. *DIGESTION OF LIPIDS:*

A. **Digestion of triacylglycerols**:

Triacylglycerols are digested by a group of enzymes. These are lingual, gastric, pancreatic and intestinal lipase enzymes.

1. Lingual lipase:

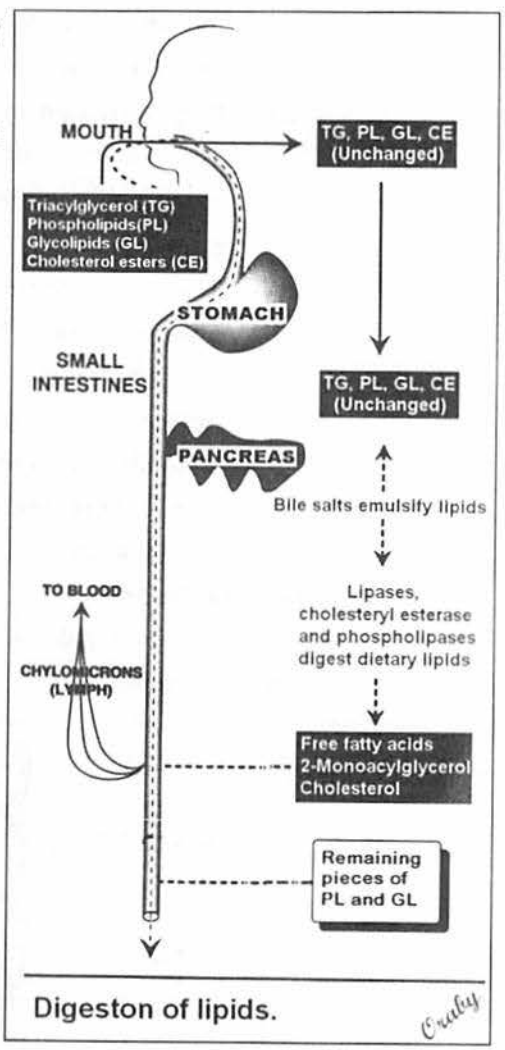
a) Secreted by Ebner's glands on the dorsal surface of the tongue.

b) Because food remains for a short time in the mouth, digestion of triacylglycerols by lingual lipase is minimal.

2. Gastric lipase:

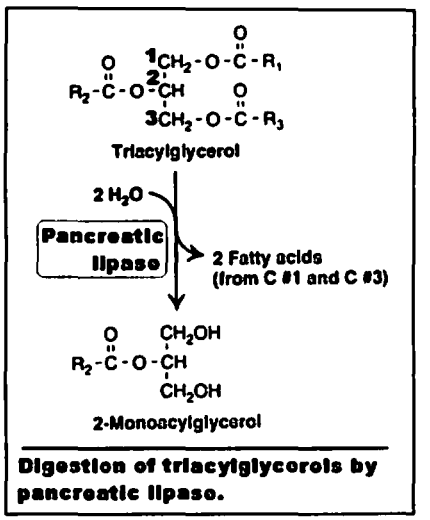
a) Optimum pH for gastric lipase is 7. Thus it cannot act in adult stomach (pH: 1-2).

b) Gastric lipase may be of value in infants stomach (pH: 5). It acts on milk fats.



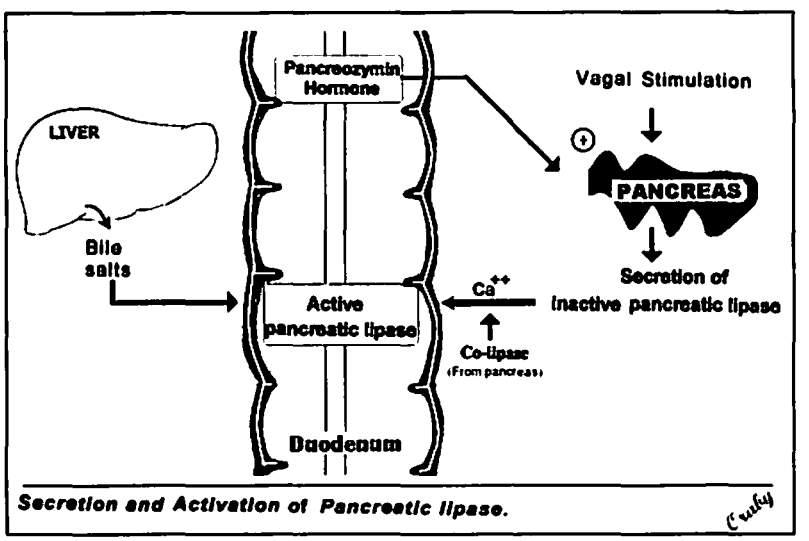
Digeston of lipids.

3. **Pancreatic lipase:**
   - *a)* It is the most important lipase in digestion of triacylglycerols.
   - *b)* It attacks the primary ester bonds of TG (position 1 & 3), hydrolyzing them into fatty acids and 2-monoacylglycerols.
   - *c)* **The resulting 2-monoacylglycerols will undergo:**
     1) 72% are absorbed as such.
     2) 28% are converted into 1-monoacylglycerols by isomerase enzyme which are then :
        - i- Absorbed as 1-monoacylglycerols (6%)
        - ii- Hydrolyzed by pancreatic lipase into glycerol and fatty acids (22%) which are then absorbed.
   - *d)* **Pancreatic lipase is secreted as inactive enzyme:**
     1) Its secretion is stimulated by pancreozymin hormone (secreted by the duodenum) and vagus nerve stimulation.
     2) It is activated in the duodenum by bile salts, calcium ions and colipase (protein produced by the pancreas).
     3) The presence of emulsifying agents as bile salts and phospholipids is important for action of pancreatic lipase. Emulsification means breakdown of large fat globules into small ones. This increases the surface area of lipids exposed to lipase enzyme.



Digestion of triacylglycerols by pancreatic lipase.

4. **Intestinal lipase:**
   - *a)* It acts on 1-monoacylglycerols converting them into glycerol and free fatty acids.

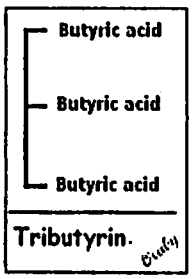


Secretion and Activation of Pancreatic lipase.

The end products of triacylglycerols digestion are:
- 72%: 2-monoacylglycerols + Fatty acids.
- 6% : 1-monoacylglycerols.
- 22%: Glycerol and fatty acids.

B. Digestion of cholesterol and tributyrin:

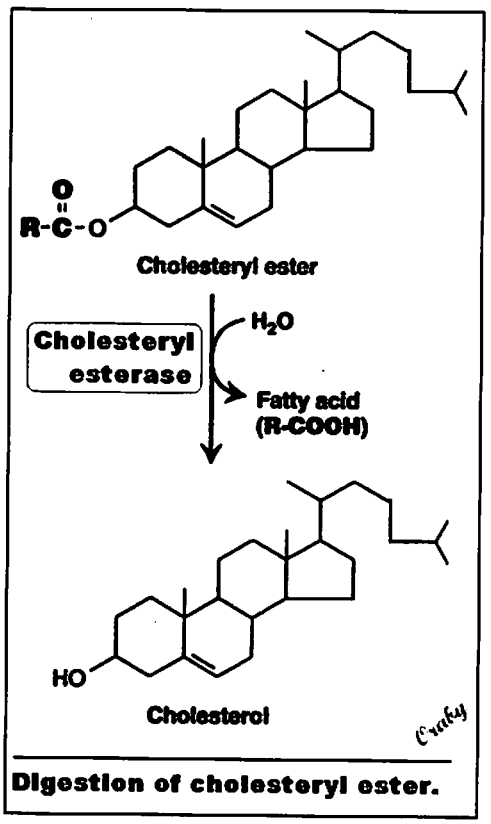
1. Cholesterol itself undergoes no digestion and absorbed as such.
2. Cholesteryl esters are digested by pancreatic cholesterol esterase enzyme into cholesterol and fatty acids.
3. Cholesterol esterase enzyme can also act on tributyrin (triacylglycerols containing 3 butyric acids) hydrolyzing them into glycerol and 3 butyric acid molecules. Milk is rich in tributyrin.



Tributyrin.

C. Digestion of phospholipids:

1. Phospholipids may be absorbed as such or digested by phospholipase enzymes (A_1, A_2 (B), C and D)
2. They act on phospholipids hydrolyzing them into fatty acids, glycerol, phosphate and nitrogenous bases.

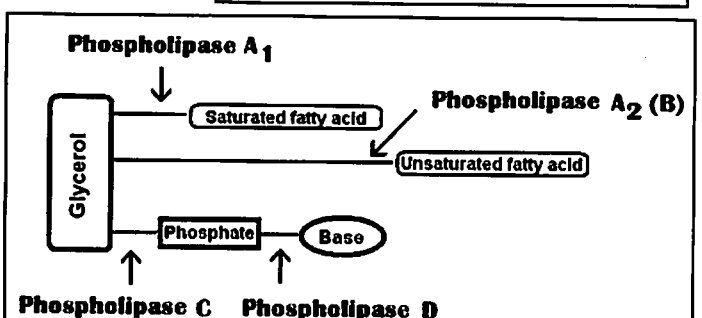


Digestion of cholesteryl ester.

IV. *ABSORPTION OF LIPIDS:*

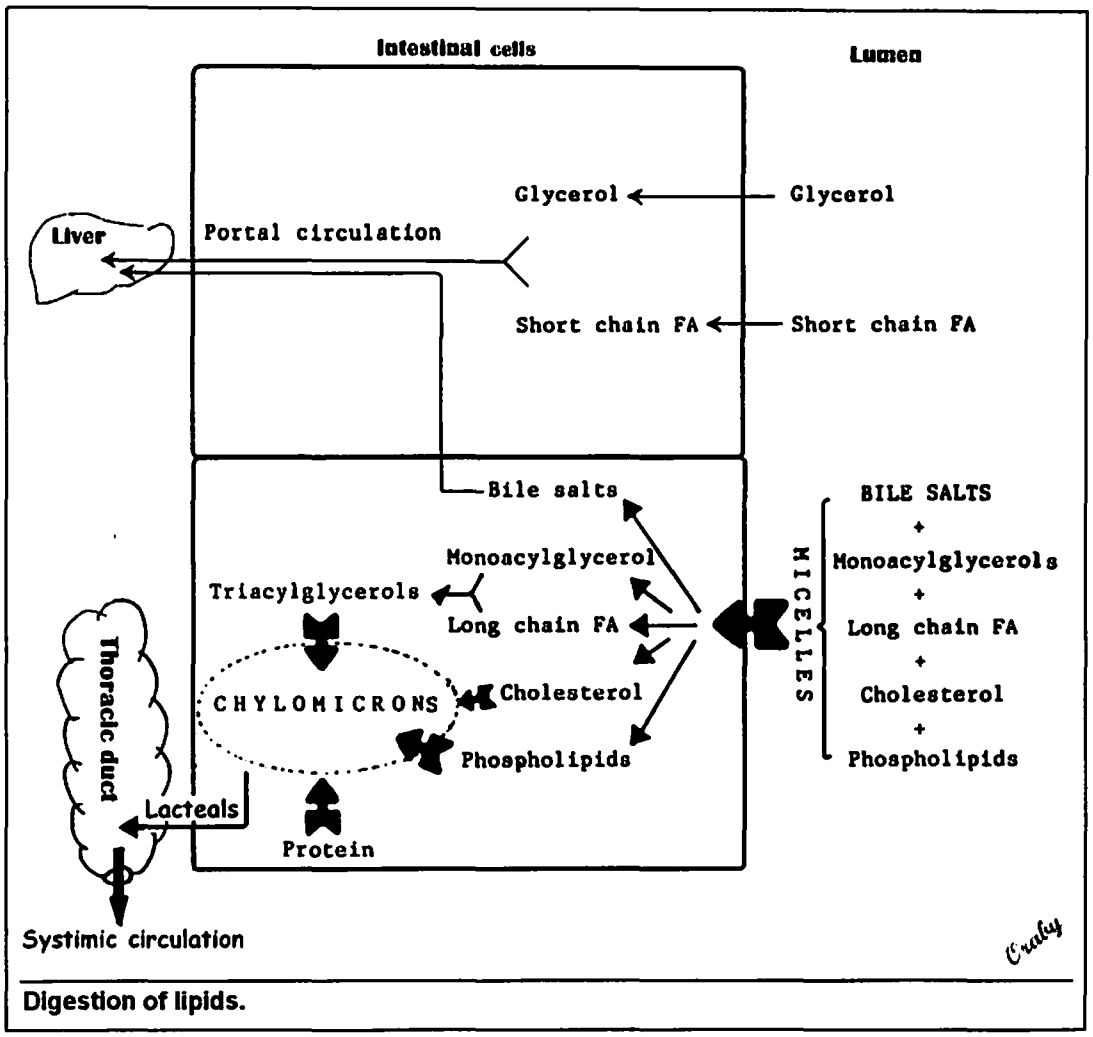
A. Mechanism:

1. **The end products of lipids digestion are** monoacylglycerols, fatty acids [short chains & long chains], glycerol, phospholipids, and cholesterol. They are absorbed from **jejunum and ileum.**
2. Short chain fatty acids (less than 12 carbons) and glycerol are water-soluble and pass via portal circulation to the liver.
3. Other lipids are water insoluble. They combine with bile salts to form a water-soluble complex called **micelles**, which enter the mucosal cells.



Digestion of phospholipids.

4. Bile salts are reabsorbed to the liver again **(enterohepatic-circulation).**
5. Long chain fatty acids are **activated** in the mucosal cells and combine with mono & diacylglycerols to form triacylglycerols again.
6. Triacylglycerols, phospholipids and cholesterol are bound to a protein called apolipoprotein B_{48} to form chylomicrons which enter the circulation through lymphatic vessels.
7. In the blood; chylomicrons are bound with other 2 proteins (apolipoproteins E & C).
8. Chylomicrons will be metabolized as discussed later.



Digestion of lipids.

B. Errors of lipids digestion and absorption:

1. Steatorrhoea :

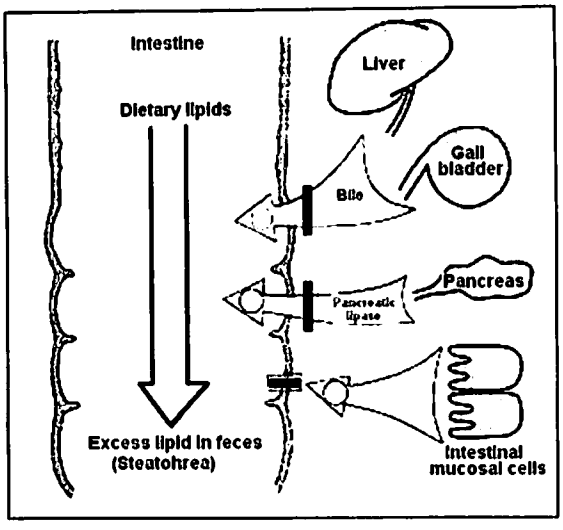
a) **Definition:** It is a condition in which fat content of the stool is abnormally increased. Normally it is less than 5 grams per day.

b) **Causes:** Steatorrhoea results from deficiency of any factor essential for digestion or absorption of lipids as pancreatic lipase, bile salts or healthy intestinal mucosa:

1) **Deficiency of pancreatic lipase :**

i- It is due to **pancreatic duct obstruction, pancreatitis** (inflammation of pancreas) or **Zollinger Ellison disease** (where the high acid content of stomach enters the duodenum and inactivates lipase enzyme.

ii- The fat in stool is undigested. There is no loss of fat-soluble vitamins because vitamins need no digestion.



2) **Deficiency of bile salts :**

i- It occurs due to **bile duct obstruction** (by stone or tumor) or **liver disease** (as in cirrhosis).

ii- The fat in stool is digested and there is loss of fat soluble vitamin (as they need bile salts for absorption).

3) **Defective cells of intestinal mucosa.**

2. Chyluria (milky urine):

a) It is the presence of fat (chylomicrons) in urine after fatty meal.

b) It is due to abnormal connection between the lymphatic drainage of the intestine and the urinary system e.g. as in elephantiasis.

V. *FATE OF ABSORBED LIPIDS:*

A. <u>After fatty meal,</u> plasma shows a milky appearance. This is due to venous blood contains excess chylomicrons after absorption.

1. Excess chylomicrons stimulate mast cells to produce heparin. Heparin then stimulates the lining epithelium of blood vessels of heart, lungs, spleen and adipose tissue to produce an enzyme called: lipoprotein lipase (plasma clearing factor).

2. <u>Lipoprotein lipase enzyme</u> (after being activated by a protein called apolipoprotein C II) will act on triacylglycerols of chylomicrons, converting them into glycerol and free fatty acids.

B. Glycerol and fatty acids are taken up by different tissues for the following fate:

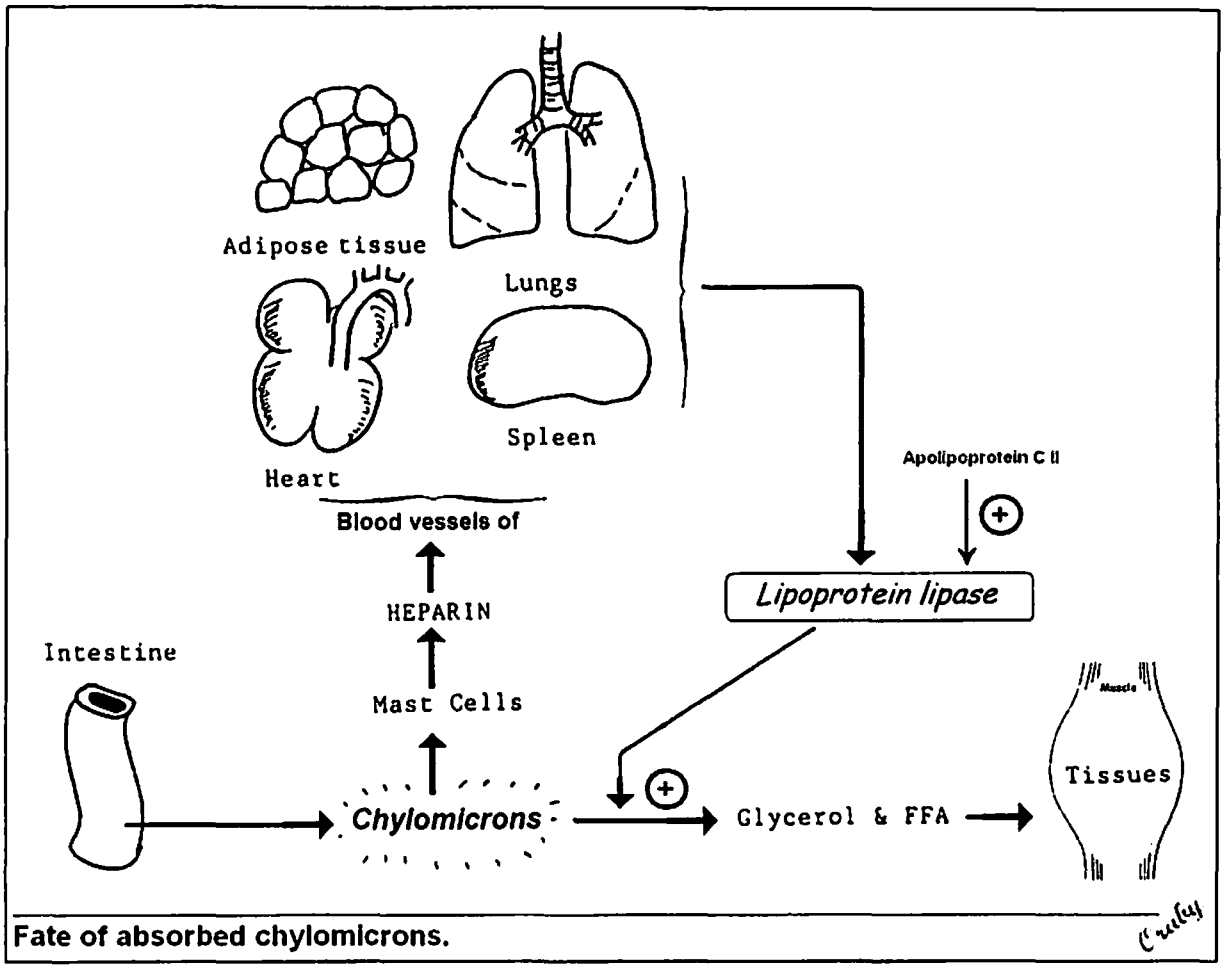
1. **Formation of depot fat (adipose tissue).**
   a) Adipose tissue is formed mainly of triacylglycerols.
   b) It is present in fat cells of adipose tissue.

2. **Oxidation for production of energy:**

3. **Glucose formation by gluconeogenesis:**
   a) Glycerol → Glucose.

4. **Synthesis of biologically active compounds:** e.g. different steroids and eicosanoids (prostaglandins, prostacyclin, thromboxanes and leuckotrienes).

5. **Synthesis of tissue fats (structural cellular fats):** They include mainly phospholipids and cholesteryl esters.



Fate of absorbed chylomicrons.

STORAGE AND MOBILIZATION OF LIPIDS

I. ***Types of body lipids:*** Body lipids are of 4 types; tissue lipids, adipose tissue (depot fat), plasma lipoproteins and bone marrow lipids.

A. **Tissue lipids:**

1. These lipids enter in the structure of body cells as cell membrane and mitochondria.
2. Tissue lipids are never oxidized to give energy.

B. **Adipose tissue (depot fat):** It is of 2 types white and brown.

1. White adipose tissue:

a) **Composition:**

1) Triacylglycerols: main content that contain saturated and unsaturated fatty acids. This makes depot fat in a fluid state at body temperature.
2) Little phospholipids and cholesterol.

b) **Site:**

1) Under skin (subcutaneous fat) and in the breast.
2) Around important organs e.g. kidney.
3) In the omentum and mesentry.

c) **Sources:**

1) Absorbed fat.
2) Carbohydrate (by lipogenesis).

d) **Functions:** depot fat is important for:

1) **Energy production:** During fasting, the triacylglycerols stored in depot fat provide the body with free fatty acids that are oxidized to give energy.
2) **Fixation of some organs e.g. kidney.**
3) **Heat insulator around the body.**
4) **Production of vitamin D_3:** Exposure of skin to ultraviolet rays of sun transforms 7-dehydrocholesterol into vitamin D_3.

2. Brown adipose tissue:

a) Certain areas of adipose tissue appear brown in color as they contain high content of mitochondria, cytochromes and well developed blood supply.

b) The brown adipose tissue may have a special function in the production of heat. They contain a protein called **thermogenin.** This protein acts as uncoupler of oxidative phosphorylation ➔ ↓ ATP production and ↑ heat generation.

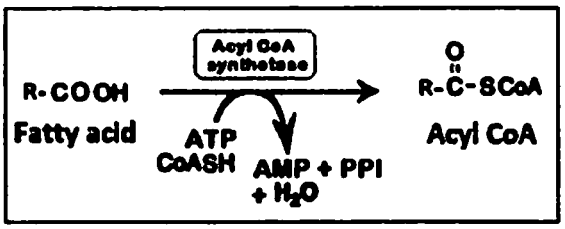
c) Brown adipose tissue is common in animals exposed to cold atmosphere for warmness. It is little in human, especially in obese persons.

Two processes control the amount of triacylglycerols in depot fat: lipogenesis and lipolysis

II. *Lipogenesis:*

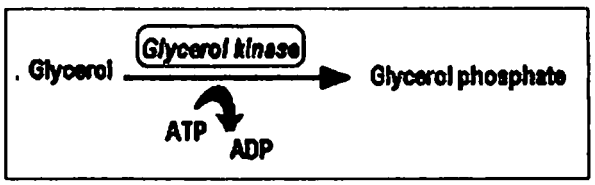
A. **Definition:** Lipogenesis is the synthesis of triacylglycerols from fatty acids (acyl CoA) and glycerol (glycerol-3-phosphate).

1. **Activation of fatty acids into fatty acyl CoA:**



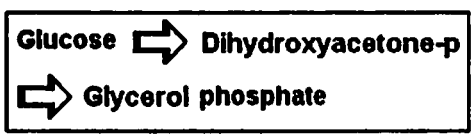
2. **Synthesis of glycerol phosphate :**

a) **In liver, kidney, intestine, and lactating mammary glands:** Glycerol phosphate is formed from glycerol by **glycerokinase** or from glucose through

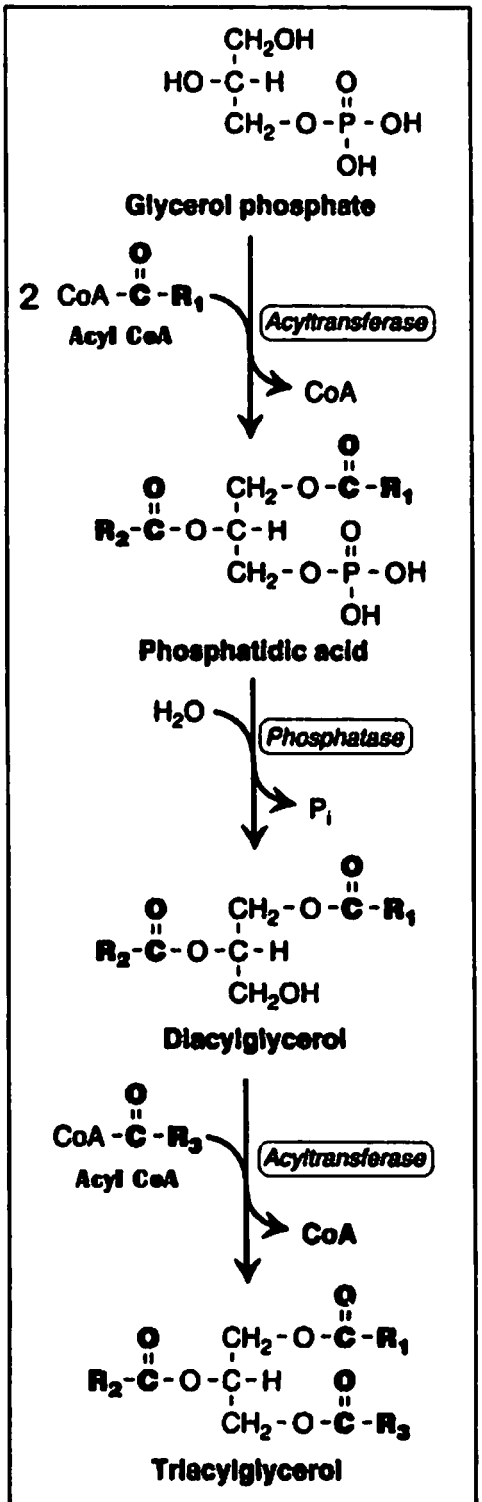


glycolysis.

b) **In muscles and adipose tissue, glycerokinase is deficient. In these tissues glycerol phosphate is formed from glucose (through glycolysis) as follows:**



3. **Synthesis of triacylglycerol:** by reaction between acyl CoA and glycerol phosphate as shown in the diagram.



Synthesis of triacylglycerols.
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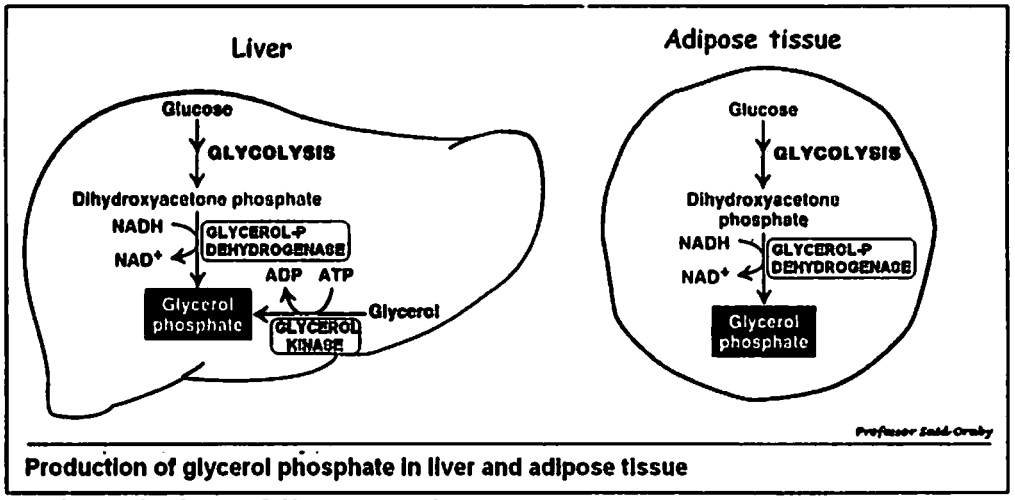


Production of glycerol phosphate in liver and adipose tissue

B. Regulation of lipogenesis:

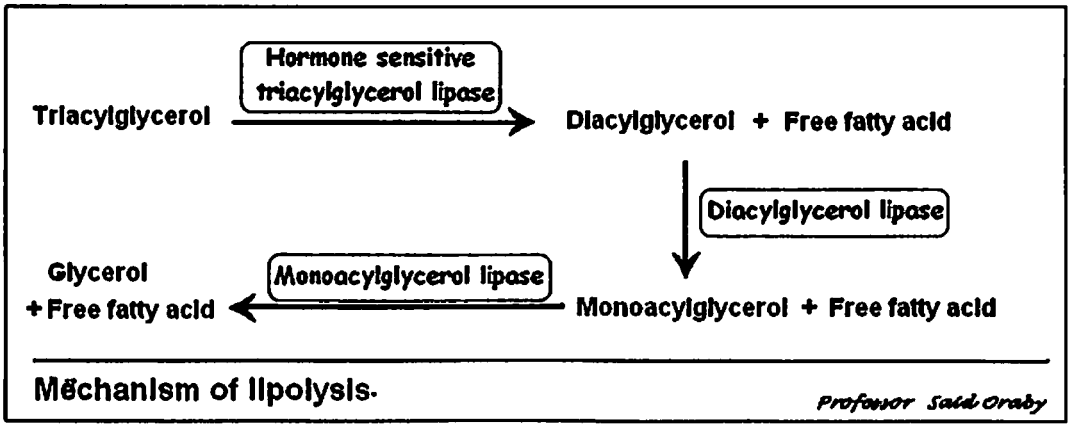
1. **After meal, lipogenesis is stimulated:** Insulin is secreted which stimulates glycolysis. Glycolysis supplies dihydroxyacetone phosphate that is converted into glycerol phosphate in adipose tissue.
2. **During fasting lipogenesis is inhibited,** as anti-insulin hormones e.g. glucagon are secreted. These inhibit lipogenesis and stimulate lipolysis.

III. *LIPOLYSIS :*

A. Definition and location: Lipolysis is the hydrolysis of triacylglycerols into glycerol and fatty acids. Its location is the cytosol of adipose tissue cells.

B. Steps: Lipolysis is carried out by a number of lipase enzymes, which are ***present in adipose tissue.*** These are :

1. Hormone sensitive triacylglycerol lipase.
2. Diacylglycerol lipase.
3. Monoacylglycerol lipase.



C. Fate of products of lipolysis:

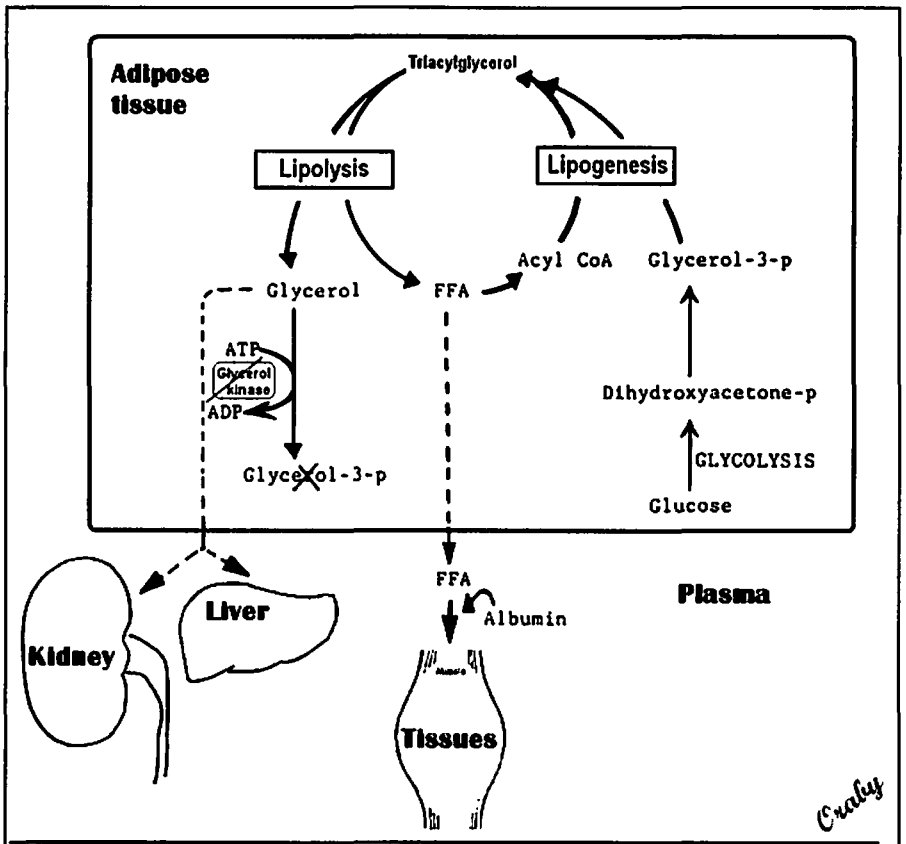
1. **Fate of fatty acids:**
   a) Oxidation by tissues to give energy.
   b) Fatty acids may remain in adipose tissue to be re-esterified into triacylglycerols again.

2. Fate of glycerol:

a) Glycerol may diffuse to blood and then taken up by the liver to give:

1) Glucose by gluconeogenesis.
2) Pyruvate by glycolysis.
3) Triacylglycerols by lipogenesis (outside adipose tissue).

> **Note:** In adipose tissue, glycerokinase enzyme is deficient, which is essential to convert glycerol into glycerol phosphate. Thus glycerol in adipose tissue cannot be used in re-esterification of fatty acids to form triacylglycerols.



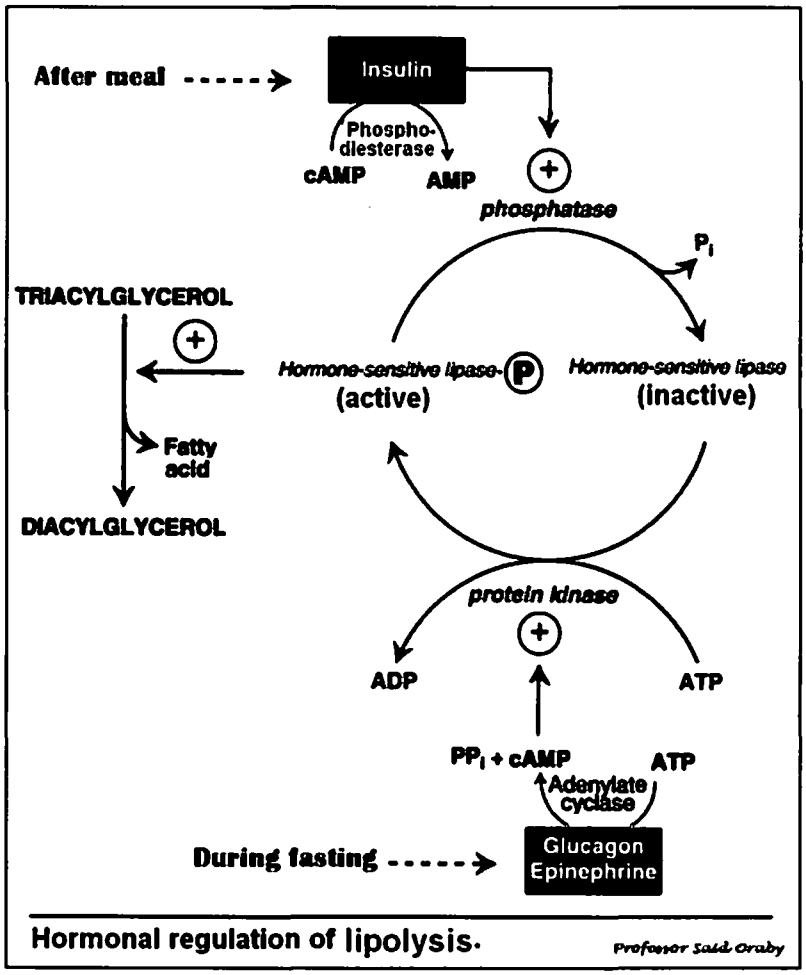
Lipolysis and fate of its products.

D. <u>Regulation of lipolysis</u>: The key enzyme controlling Lipolysis is the hormone sensitive triacylglycerol lipase. It exists in 2 forms: active (phosphorylated) and inactive (dephosphorylated)

1. During fasting:

a) Many hormones (epinephrine, norepinephrine and glucagon) are secreted. They stimulate adenylate cyclase enzyme ➔ formation of cAMP ➔ Activation of protein kinase enzyme ➔ Phosphorylation of hormone sensitive triacylglycerol lipase ➔ Stimulation of lipolysis.

2. **After meal:**
   a) Insulin is secreted ➔ Stimulation of both phosphodiesterase enzyme and lipase phosphatase enzyme ➔ dephosphorylation and inactivation of hormone sensitive triacylglycerol lipase enzyme ➔ Inhibition of lipolysis
   b) Caffeine is a substance present in coffee. It inhibits phosphodiesterase enzyme ➔ Stimulation of lipolysis.



E. **Causes of excessive lipolysis:** In conditions where the need for energy is increased (low insulin and high glucagon):
   1. Starvation.
   2. Diabetes mellitus.
   3. Low carbohydrate diet.
   4. Hypercatabolic states e.g. fevers, hyperthyroidism.

Fatty acids oxidation

Among the different foodstuffs, lipids give (through fatty acids oxidation) the maximum amount of energy. 3 Different pathways for fatty acid oxidation are present: α, β and ω.

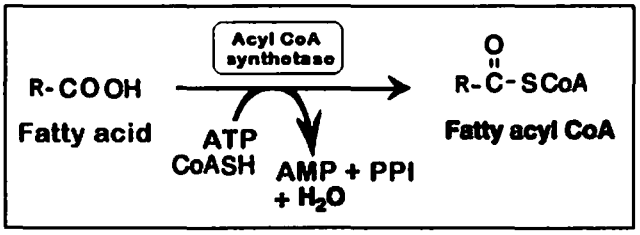
I. *β-Oxidation:*

A. Site:

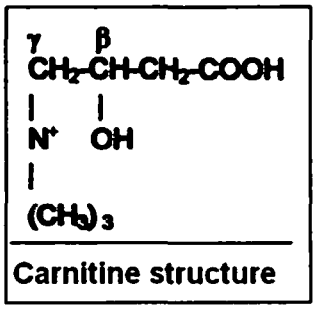
1. **Intracellular location:** Mitochondria.
2. **Organ location:**
   a) Liver, kidney, heart and skletal muscles.
   b) β-Oxidation never occur in brain and RBCs.

B. Transport of fatty acids into the mitochondria:

1. After the fatty acid is taken up by the cell, it is converted to the acyl CoA in the cytosol:



2. Because the β-oxidation occurs in the mitochondrial matrix, the acyl CoA must be transported across the mitochondrial inner membrane, which is impermeable to acyl CoA. Therefore, a specialized carrier present in the inner mitochondrial membrane called **carnitine**, transports the acyl CoA from cytosol into the mitochondrial matrix. The transport process is called carnitine shuttle.
3. **Carnitine shuttle:**
   a) **Structure of carnitine:**
   Carnitine is β-hydroxy-γ-trimethyl ammonium butyric acid. It is derived from lysine amino acid.



Carnitine structure

   b) **Function of carnitine:**
   It transports long chain acyl CoA inside the mitochondrial matrix where enzymes for β oxidation are present.
   c) **Steps of shuttle:** Three enzymes are involved:
      1) **Carnitine acyl transferase I:**
         i- For palmitic acid, it is called carnitine palmitoyl transferase I
         ii- It is present in the outer mitochondrial membrane.

iii- It transfers the acyl group from acyl CoA to carnitine to form acyl carnitine.

2) **Carnitine acylcarnitine translocase:**

i- It is present in the inner mitochondrial membrane.

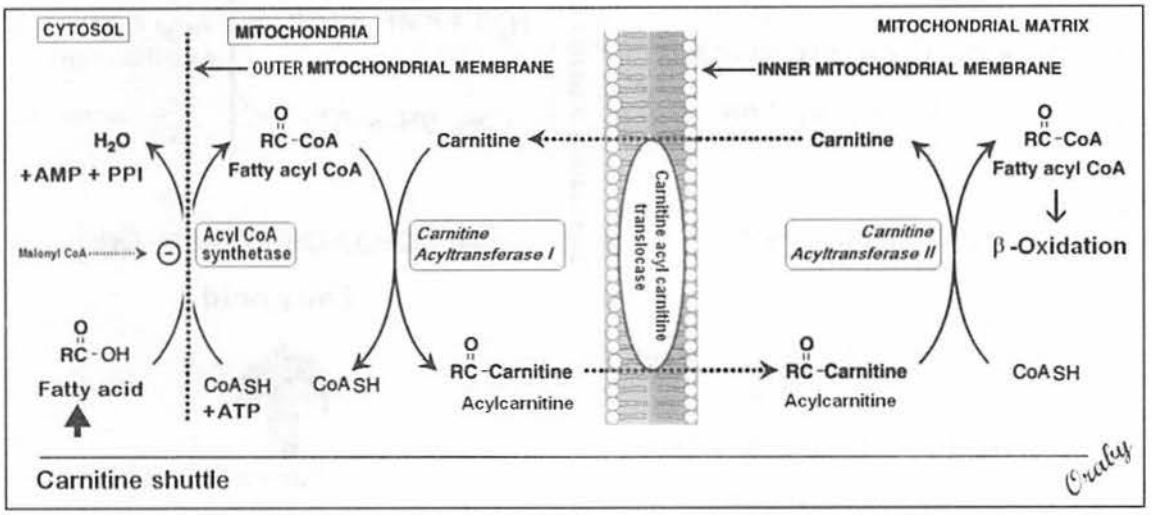
ii- It transports acyl carnitine across inner mitochondrial membrane (in exchange with carnitine).

3) **Carnitine acylcarnitine transferase II:**

i- For palmitic acid, it is called carnitine palmitoyl transferase II.

ii- It is present in the inner mitochondrial membrane.

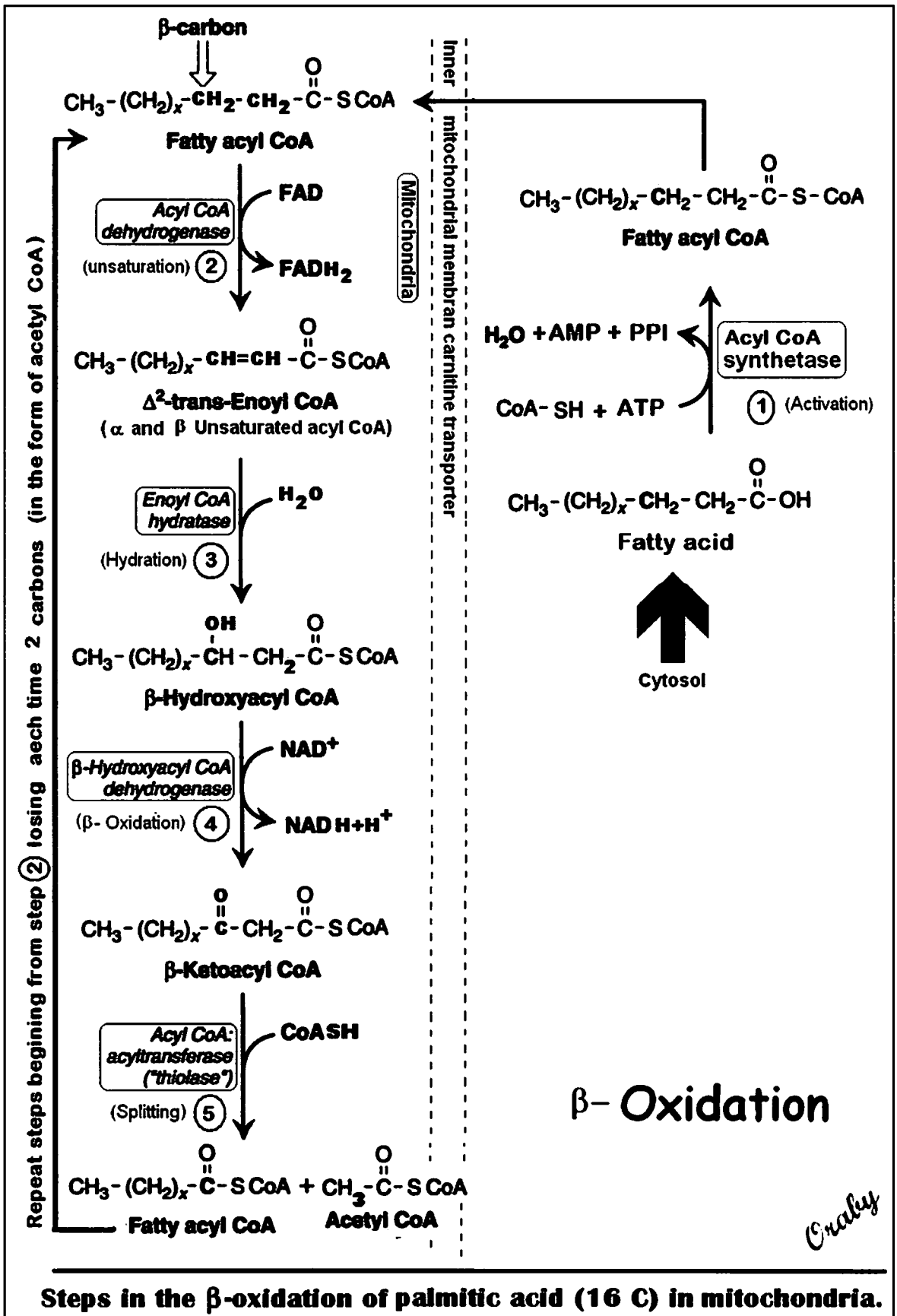
iii- It transfers the acyl group from acyl carnitine to form acyl CoA again.



C. Steps of β-Oxidation:

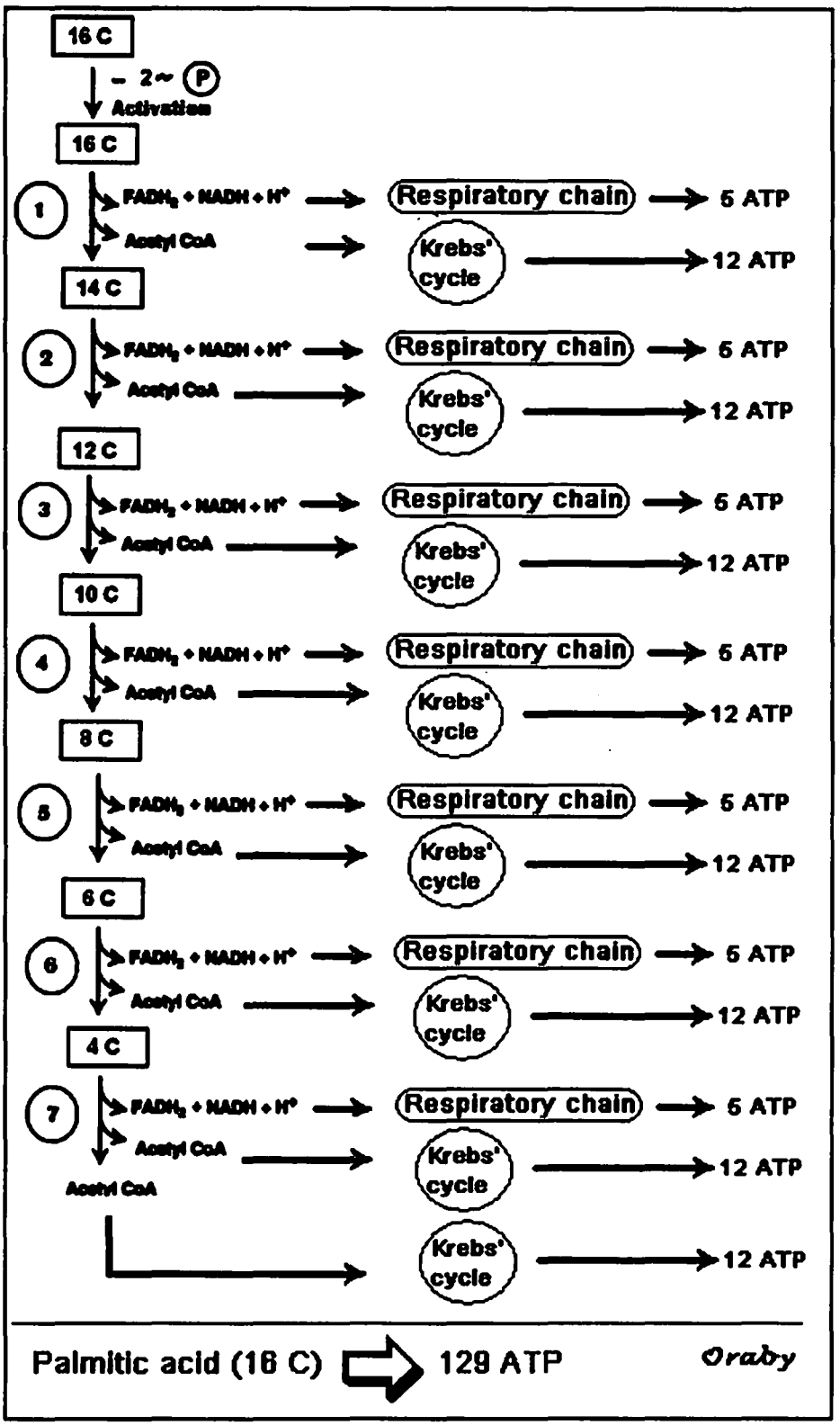
1. **Activation of fatty acid and transport inside the mitochondria:**
   - *a)* Catalyzed by fatty acyl CoA synthetase enzyme and carnitine shuttle.
   - *b)* 2 high energy bonds (~P) are utilized: ATP ➔ AMP + PPi.
2. **Unsaturation of fatty acids:**
   - *a)* Catalyzed by fatty acyl CoA dehydrogenase enzyme.
   - *b)* This enzyme is one of flavoproteins and its reduced coenzyme ($FADH_2$) gives 2 ATP when oxidized in the respiratory chain.
3. **Hydration:**
   - *a)* Catalyzed by enoyl CoA hydratase enzyme.
   - *b)* It helps the addition of water to saturate double bonds.
4. **Oxidation:**
   - *a)* Catalyzed by β-hydroxyacyl CoA dehydrogenase enzyme.
   - *b)* Its reduced coenzyme ($NADH+H^+$) gives 3 ATP when oxidized in the respiratory chain.
5. **Splitting (cleavage) step:**
   - *a)* Catalyzed by Acy CoA acyl transferase (thiolase) enzyme.

b) It splits acyl CoA into acetyl CoA and acyl CoA (shorter than the original one by 2 carbon atoms).



Steps in the β-oxidation of palmitic acid (16 C) in mitochondria.

D. Energy production of β-oxidation:



1. e.g. palmitic acid (16 carbons):
   a) β-oxidation of palmitic acid will be repeated 7 times (turns) to produce 8 acetyl CoA.

b) In each turn, one molecule of reduced $FADH_2$ and one molecule of reduced $NADH+H^+$ are produced. They are oxidized in respiratory chain to give 5 ATP.

$FADH_2$ ➔ 2 ATP

$NADH+H^+$ ➔ 3 ATP

∴7 turns × 5 ATP → 35 ATP.

c) Oxidation of one molecule of acetyl CoA in Krebs' cycle gives 12 ATP.

∴8 Acetyl CoA × 12 ATP = 96 ATP

d) Two high energy phosphate bonds are utilized in the first reaction (catalyzed by acyl CoA synthetase) which occurs for one time only.

e) **∴ Net energy gain =**

Energy produced – Energy utilized

=(35 ATP + 96 ATP) – 2 ATP

=131 ATP – 2 ATP = 129 ATP

2. **Calculation formula of energy production of oxidation of any fatty acid:**

$$= \left[\left(\frac{N}{2}-1\right)x\,5\,ATP\right]+\left[\frac{N}{2}x\,12\,ATP\right]-2\,ATP$$

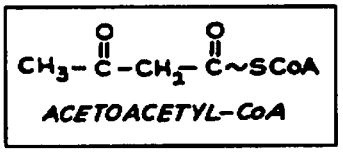
Where N = Number of carbons of fatty acid e.g. palmitic acid = 16 carbons, so energy production =

$$= \left[\left(\frac{16}{2}-1\right)x\,5\,ATP\right]+\left[\frac{16}{2}x\,12\,ATP\right]-2\,ATP$$

={(8 – 1) × 5 ATP} + {8 x 12 ATP} – 2 ATP = 129 ATP

E. Importance (functions) of β-oxidation:

1. **Energy production** e.g. palmitic acid produces 129 ATP.
2. **Production of acetyl CoA** which enter in many pathways (see sources and fate of acetyl CoA).
3. **Ketone bodies formation**: Acetoacetyl CoA is the last 4 carbons product in the course of β-oxidation of even numbered fatty acids. It may be converted into acetoacetate; one of ketone bodies (see later).



ACETOACETYL-CoA

F. β-oxidation of odd number fatty acids:

1. They are oxidized by β-oxidation till a propionyl CoA (3 carbons) is produced. Then propionyl CoA is converted to succinyl CoA as shown in the following figure.
2. **Sources and Fate of succinyl CoA:**

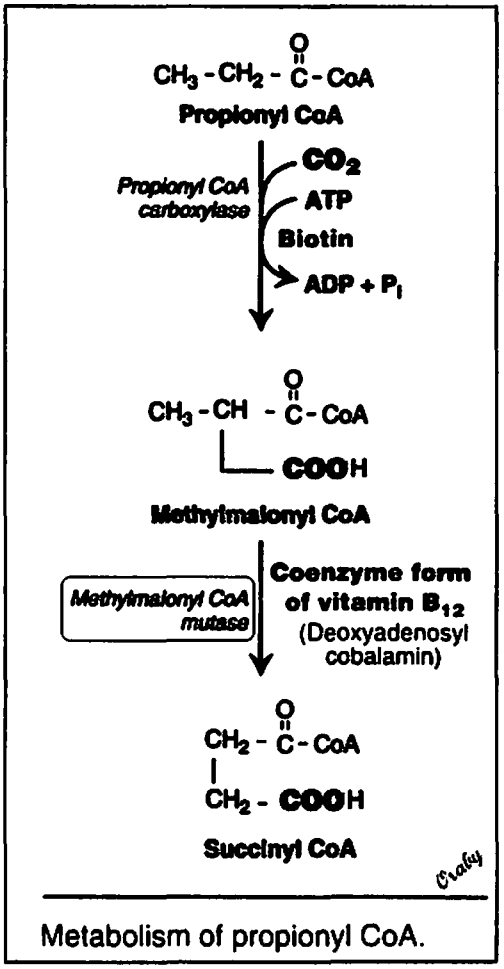
a) **Sources:**

1) Oxidation of odd number fatty acids.

2) Citric acid cycle.
3) Catabolism of some amino acids e.g. leucine, valine and methionine.

b) **Fate:**

1) Glucose synthesis (gluconeogenesis).
2) Heme synthesis.
3) Oxidation in citric acid cycle.
4) Activation of ketone bodies.
5) Detoxication.



Metabolism of propionyl CoA.

II. *α-Oxidation:*

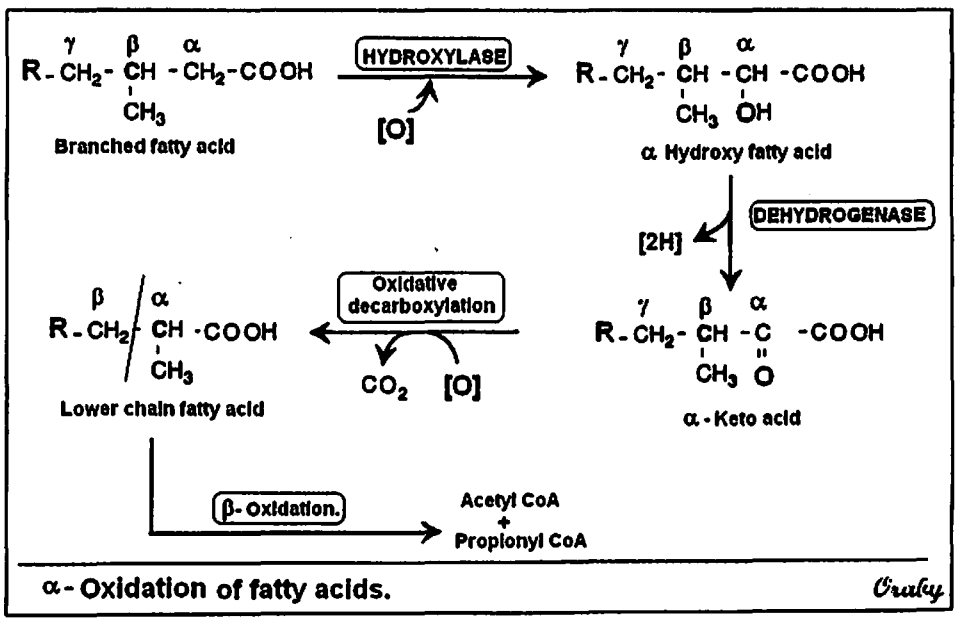
A. <u>This type of oxidation occurs in α position and characterized by:</u>

1. It is a mechanism mainly for oxidation of branched chain fatty acid, which are methylated at β position.
2. It is specific for oxidation of phytanic acid (3, 7, 11, 15 tetramethyl palmitic acid), present in plant foodstuffs.
3. It is minor pathway for fatty acid oxidation, occurs mainly in brain and nervous tissues.

B. In α-oxidation, there is one carbon atom removed at a time from α position.

C. It does not require CoASH and does not generate high energy phosphate.

D. <u>Steps:</u>



α-Oxidation of fatty acids.

E. **Refsum's disease:**

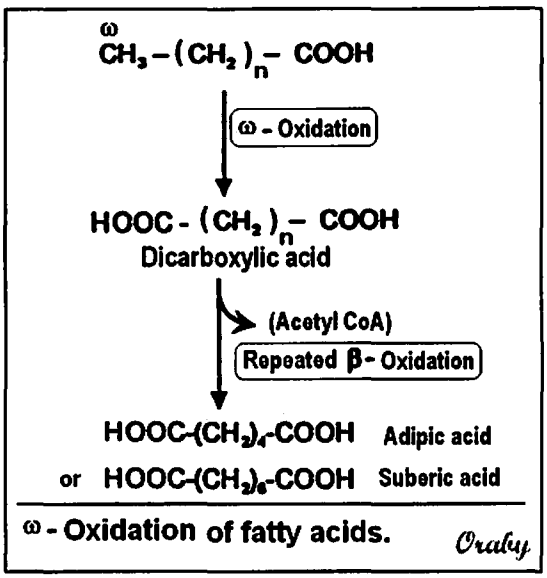
1. **This is inherited deficiency of enzymes responsible for** α oxidation of phytanic acid. This leads to accumulation of phytanic acid in nervous tissue and produce nervous damage e.g. deafness and blindness.

III. ω-Oxidation:

A. It is oxidation of **terminal CH_3** group (ω carbon) of fatty acid.

B. This produces dicarboxylic fatty acids. By β oxidation they are converted to adipic acid (6 carbons) and suberic acid (8 carbons).

C. ω-Oxidation is a minor pathway for fatty acid oxidation and catalyzed by hydroxylase enzymes of cytochrome P450.



SOURCES AND FATE OF ACTIVE ACETATE (ACETYL CoA)

I. Sources:

A. **Lipids**: Oxidation of fatty acids and ketone bodies.

B. **Carbohydrate**: Glucose oxidation ➔ Pyruvate ➔ Acetyl CoA.

C. **Proteins**:

1. **Ketogenic amino acids**: Give directly acetyl CoA or indirectly give acetoacetate ➔ Acetyl CoA.
2. **Glucogenic amino acids** ➔ Pyruvate ➔ Acetyl CoA.

II. Fate:

A. **Oxidation**: Through Krebs' (citric acid cycle).

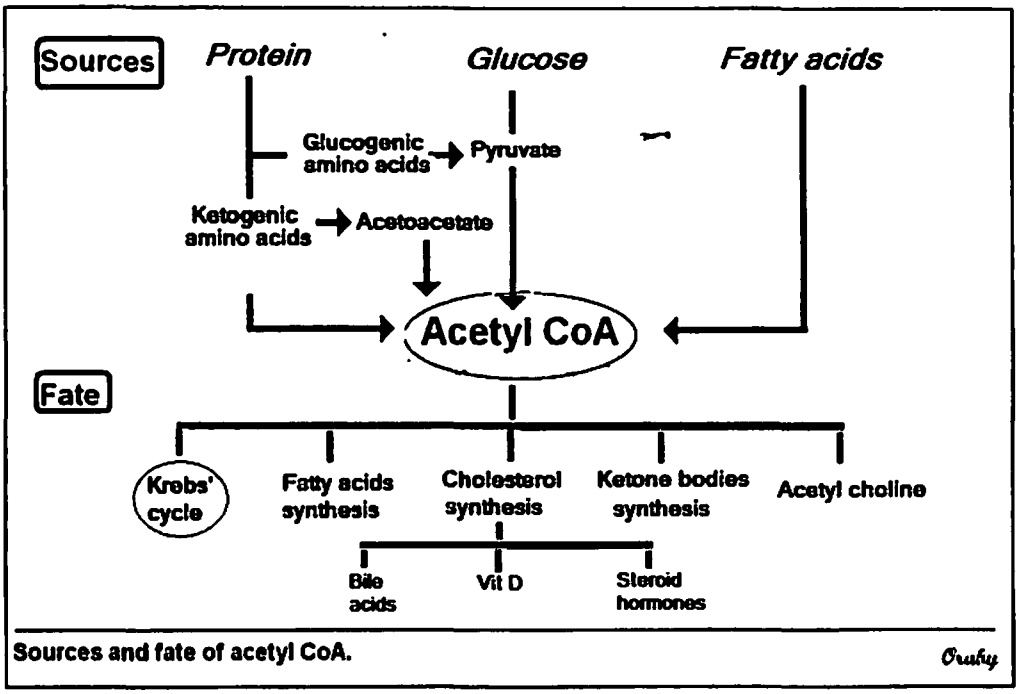
B. **Lipogenesis**: Formation of fatty acids and triacylglycerols.

C. **Ketogenesis**: Synthesis of ketone bodies.

D. **Acetylcholine synthesis.**

E. **Cholesterol synthesis**: which is the precursor for:

1. Bile acids.
2. Vitamin D_3.
3. Steroid hormones: glucocorticoids, mineralocorticoids, male sex hormones (testosterone) and female sex hormones (estrogens and progesterone).



Sources and fate of acetyl CoA.

FATTY ACIDS SYNTHESIS

I. INTRODUCTION:

A considerable number of fatty acids are derived from diet. Living organisms have the capacity to synthesize fatty acids from acetyl CoA. Any substance that gives acetyl CoA (e.g. glucose) is called lipogenic.

There are 2 mechanisms for fatty acids synthesis: cytoplasmic and microsomal.

II. CYTOPLASMIC SYSTEM FOR FATTY ACID SYNTHESIS: Also called extramitochondrial system or de novo synthesis of fatty acids. The main product of this pathway is **palmitate (16 C).**

A. **Site:**

1. **Intracellular location:** Cytosol.
2. **Organ location:** Many tissues including liver, adipose tissue, mammary gland, lung and kidney.

B. **Requirements:** This pathway needs the following substrates; acetyl CoA, $NADPH+H^+$ and group of enzymes called collectively fatty acid synthase complex.

1. **Acetyl CoA:**

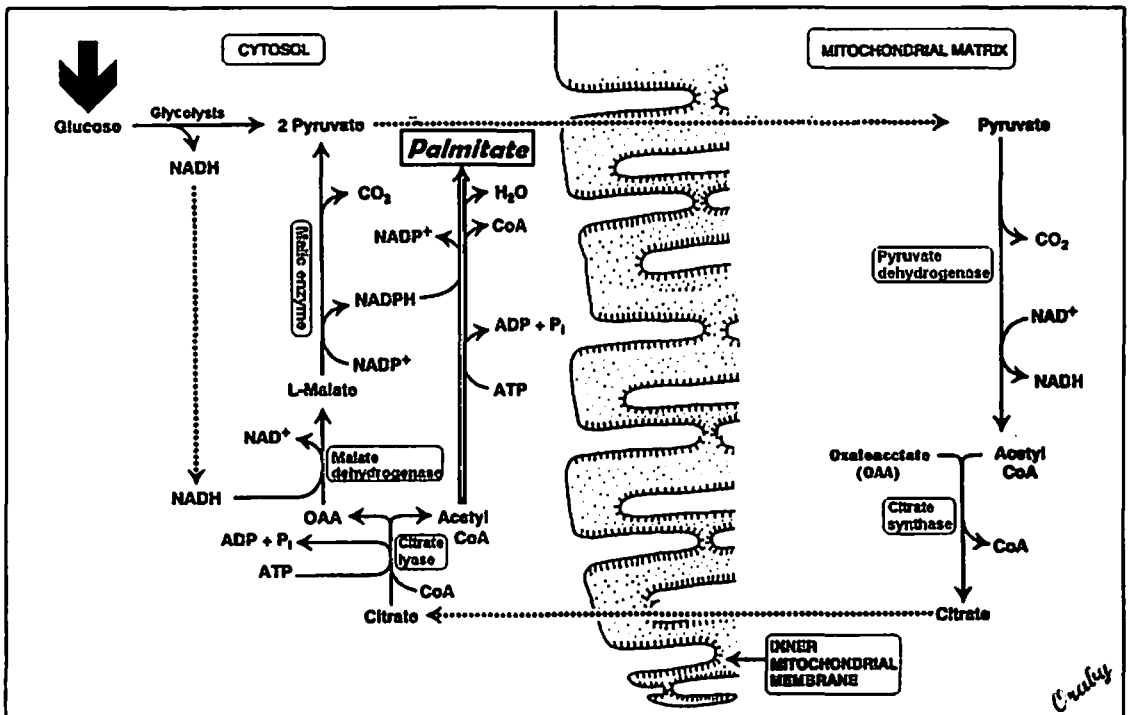
a) It is provided mainly by glucose through pyruvate (glycolysis).

b) Acetyl CoA is formed in mitochondria and fatty acid synthesis occurs in cytosol. The acetyl CoA cannot diffuse to cytosol because mitochondrial membrane is impermeable to it. Acetyl CoA condenses

with oxaloacetate – in the presence of citrate synthase- to form citrate. Then citrate diffuses out of mitochondria to cytosol where it is splitted again – by citrate lyase – into acetyl CoA and oxalaoacetate (see the following figure):

1) Acetyl CoA molecules are used for palmitate synthesis.
2) Oxaloacetate is converted to malate: Malate may be converted to pyruvate by **malic enzyme.** This reaction produces $NADPH+H^+$, which is needed for fatty acid synthesis.

Note: *Acetyl CoA used for fatty acid synthesis always derived from glucose and never from fatty acids. This is because insulin hormone secreted after meal stimulates both glucose oxidation (➔acetyl CoA) and lipogenesis (=Fatty acid synthesis) and inhibits lipolysis (➔Fatty acid oxidation➔Acetyl CoA).*



Interrelationship between glucose metabolism and palmitate synthesis. [Note: OAA= Oxaloacetate].

2. **$NADPH+H^+$:** It is provided by 3 sources:
   a) Pentose phosphate pathway.
   b) Action of cytoplasmic isocitrate dehydrogenase on isocitrate. It is similar to mitochondrial one but it uses $NADP^+$ as hydrogen carrier.

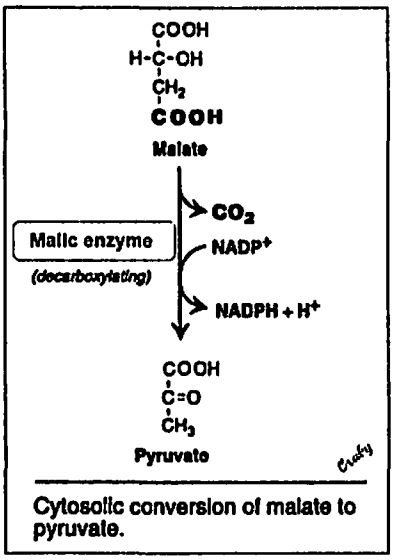
Isocitrate+$NADP^+$➔Oxalosuccinate + $NADPH+H^+$ ➔ α Ketoglutrate

   c) Action of malic enzyme on malate to produce pyruvate.
3. **Fatty acid synthase complex:**
   a) This enzyme is a **dimer** i.e. formed of 2 subunits.

b) Each unit, which is called **monomer**, contains 7 enzymes and a terminal protein called **acyl carrier protein (ACP).**

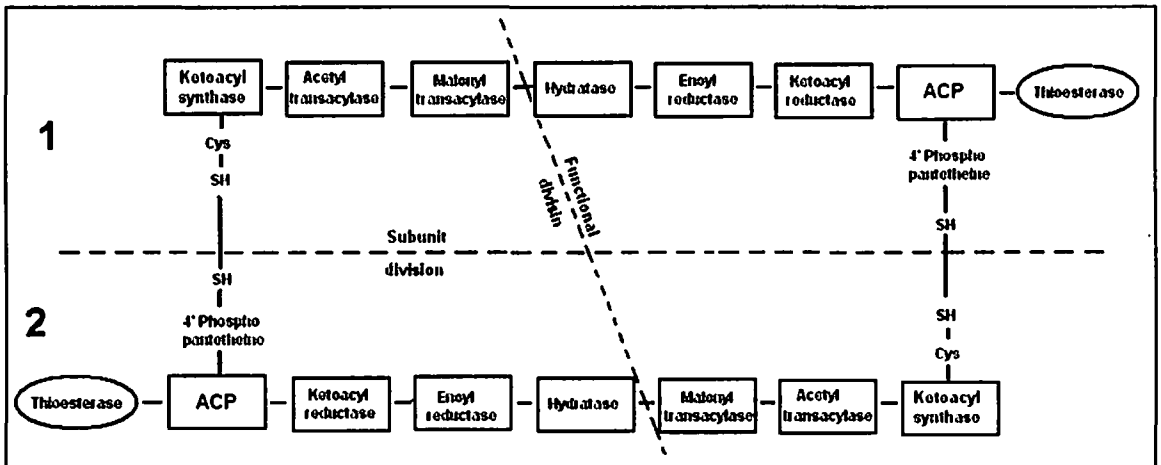
c) ACP is a protein contains the vitamin pantothenic acid in the form of phosphopantotheine. ACP is the part that carries the acyl group.

d) Each monomer contains 2 –SH groups, one provided by phosphopantotheine and attached to ACP. The other is provided by cysteine attached to the enzyme 3-ketoacyl synthase.



Cytosolic conversion of malate to pyruvate.

e) The 2 monomers are arranged head to tail, so the -SH group of ACP of one monomer is very close to the –SH group provided by 3-ketoacyl synthase of the other monomer.



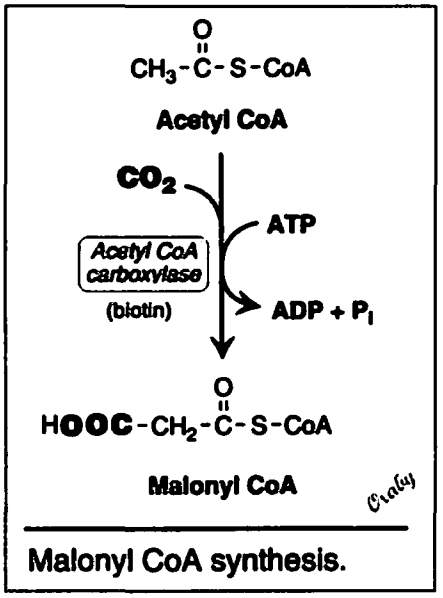
Fatty acid synthase complex. The active enzyme is a dimer of identical subunits.

C. Steps of cytoplasmic pathway:

1. **Carboxylation of acetyl CoA to form malonyl CoA:**

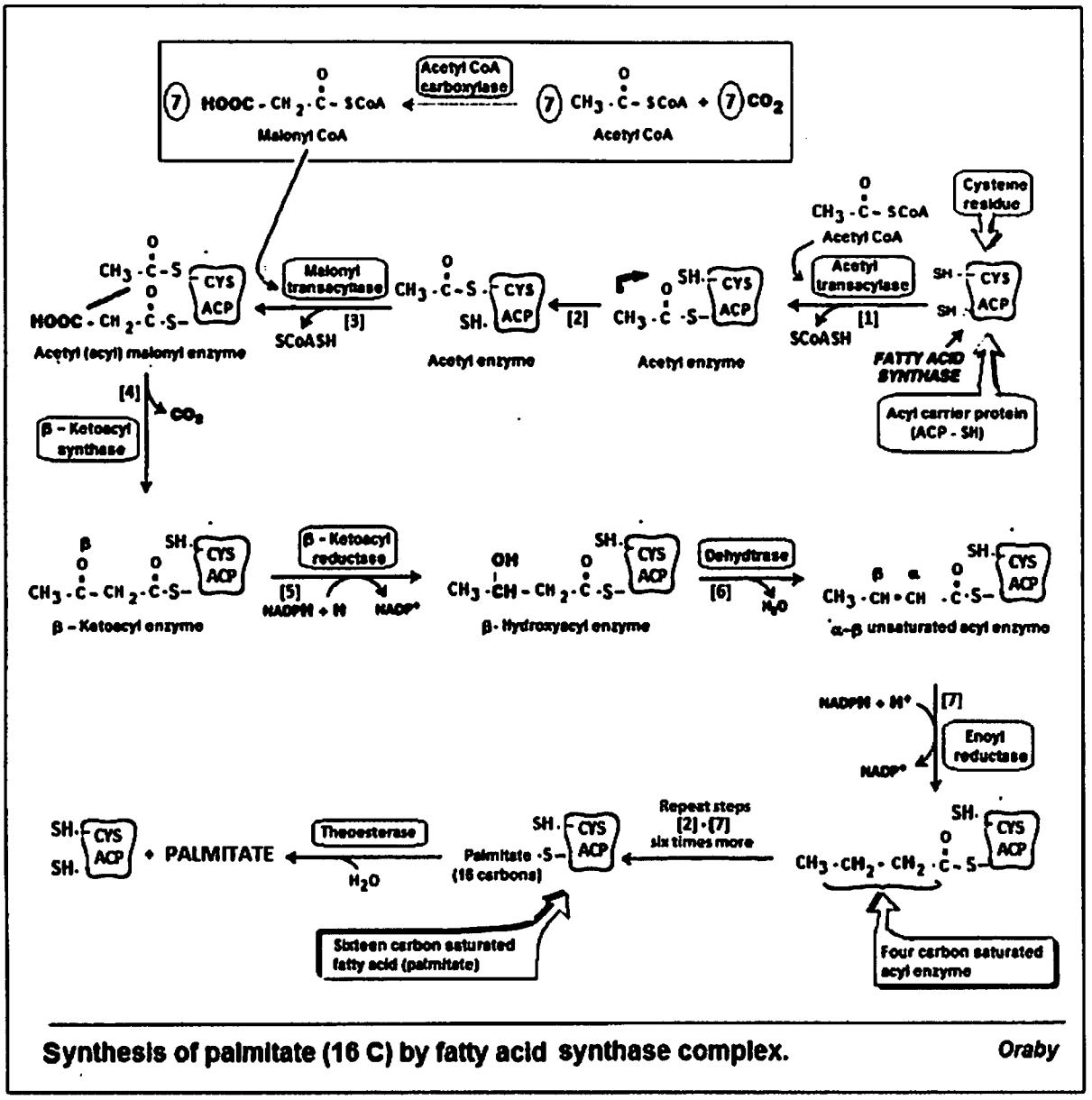
a) Malonyl CoA is synthesized from acetyl CoA by **acetyl CoA carboxylase** in the presence of biotin and ATP.

b) **It is the committed step in the pathway.**



Malonyl CoA synthesis.

2. **Synthesis of palmitate:**

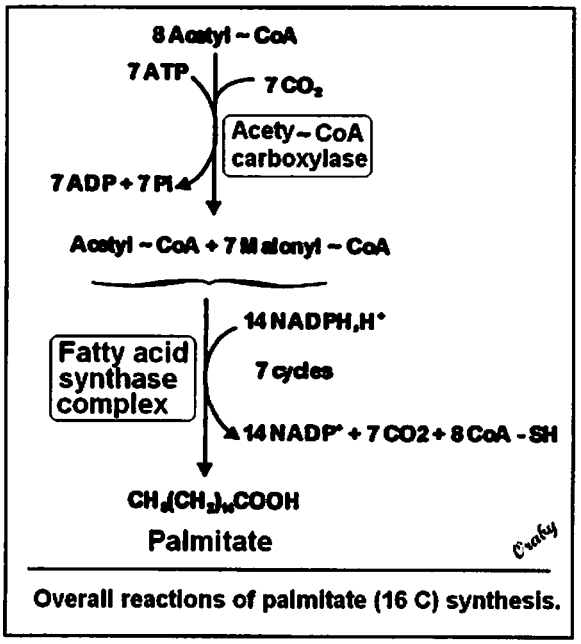


Synthesis of palmitate (16 C) by fatty acid synthase complex. *Oraby*

D. <u>Comments on synthesis of palmitate</u>:

1. A molecule of acetyl CoA combines with a pantotheine –SH group of the first monomer. This step is catalyzed by acetyl (acyl) transacylase enzyme. Then acetyl group is transferred to the cysteine –SH group of the other monomer of fatty acid synthase

2. A molecule of malonyl CoA then combines with a pantotheine –SH group of the first monomer. This step is catalyzed by malonyl transacylase enzyme. This leads to the formation of acetyl (acyl)-malonyl-enzyme.
3. Then acetyl group attacks the malonyl group to liberate CO_2 and to form 3 ketoacyl enzyme (acetoacetyl enzyme) that attached to pantotheine–SH group and to let the cysteine–SH group free (which was initially occupied by the acetyl CoA). *This decarboxylation step acts as a driving force for the whole reactions.*
4. The 3-ketoacyl group is reduced, dehydrated and then reduced again to form the corresponding acyl-enzyme (4 carbon saturated fatty acyl enzyme)
5. The 4 carbons saturated fatty acyl group is then transferred to cysteine –SH group of the other monomer of fatty acid synthase leaving the pantotheine –SH group of the first monomer for the second molecule of malonyl CoA.
6. The sequence of reactions is then repeated 6 more times to produce fatty acids of 6,8,10,12,14 and finally 16 carbons (palmitic acid).
7. Free palmitate is formed by the action of thioesterase enzyme of the multi-enzyme complex, which adds H_2O and hydrolyzes the palmitoyl enzyme.
8. **The overall reaction is as shown in the diagram:**



Overall reactions of palmitate (16 C) synthesis.

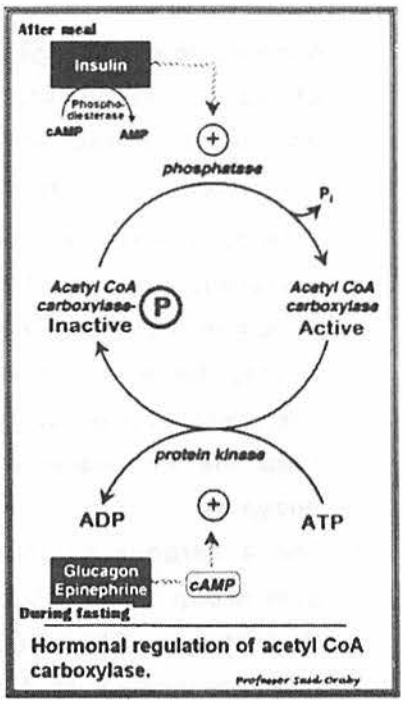
E. Fate of palmitate:

1. **Esterification:**
   Palmitate esterifieid with glycerol to from acylglycerols or with cholesterol to form cholesteryl ester.
2. **Chain elongation:**
   Palmitate may be elongated to form a longer fatty acid.
3. **Desaturation:** i.e. synthesis of unsaturated fatty acid: palmitate may undergo desaturation at C_9-C_{10} to form palmitoleic acid.
4. **Sphingosine formation:**
   It is formed by condensation of palmityl CoA and the amino acid serine.

F. **Regulation of fatty acid synthesis:**

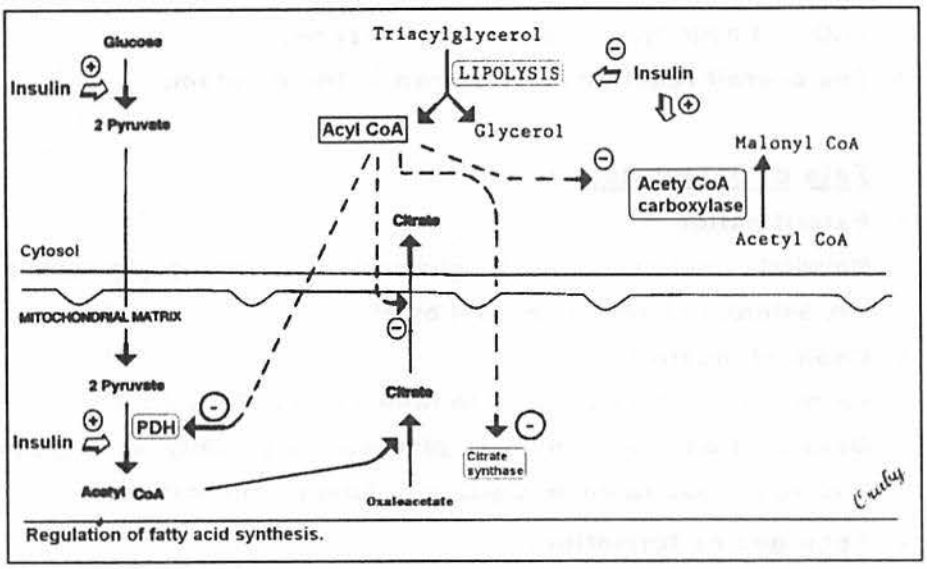
The key enzyme of cytoplasmic pathway is acetyl CoA carboxylase enzyme.

1. **Insulin:** Stimulates fatty acid synthesis through:
   a) Insulin activates both acetyl CoA carboxylase and pyruvate dehydrogenase [PDH] complex (through dephosphorylation of the enzymes).
   b) Insulin stimulates also the transport of glucose into cells e.g. adipose tissue. This increases the amount of pyruvate ⇒ Acetyl CoA ⇒ Fatty acid synthesis.
   c) Insulin inhibits lipolysis through inhibition of cAMP ⇒ No long chain fatty acids (acyl CoA) that inhibit lipogenesis.



Hormonal regulation of acetyl CoA carboxylase.

2. **Glucagon and epinephrine:** Inhibits fatty acid synthesis through cyclic AMP and it also stimulates lipolysis ➔ Long chain fatty acids ➔ inhibition of lipogenesis.
3. **Long chain acyl CoA**: Inhibits Fatty acid synthesis through:
   a) It inhibits allosterically acetyl CoA carboxylase.
   b) It inhibits transport of citrate from mitochondria to cytosol.
   c) It inhibits pyruvate dehydrogenase (PDH) that synthesizes pyruvate ➔ Acetyl CoA ➔ fatty acid.
4. **Citrate:** stimulates fatty acid synthesis through stimulation of acetyl CoA carboxylase.



Regulation of fatty acid synthesis.

	FA acid synthesis	FA acid oxidation
Organ location	Mainly liver	Mainly liver and muscles
Intracellular location	Cytosol	Mitochondria
Nutritional state	After meals	Fasting
Hormonal stimulation	Insulin	Glucagon
Carrier of acetyl/acyl group between cytosol and mitochondria	Citrate (mitochondria to cytosol).	Carnitin (cytosol to mitochondria)
Active carriers	➢ Acyl carrier protein ➢ CoASH	➢ CoASH
Coenzyme	$NADP^+$	NAD^+
Two carbon donor / product	Malonyl CoA	Acetyl CoA
Activator	Citrate	
Inhibitor	Fatty acyl CoA (inhibits acetyl CoA carboxylase)	Malonyl CoA (inhibits Carnitine acyl transferase)
Product of pathway	Palmitate	Acetyl CoA

Comparison between fatty acid synthesis (extramitochondrial pathway) and fatty acid oxidation

Professor Said Orabi

III. *MICROSOMAL PATHWAY FOR FATTY ACID SYNTHESIS:*

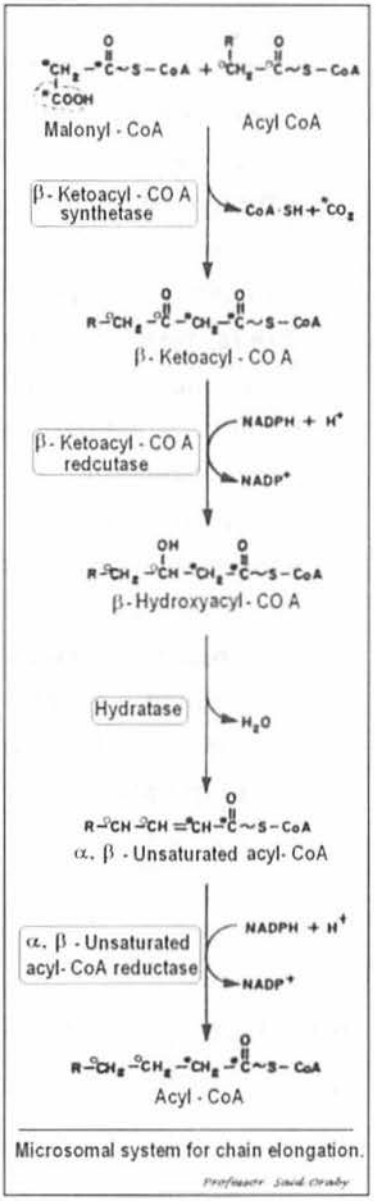
This is probably the main site for the elongation of existing long chain fatty acid molecules i.e. production of fatty acids longer than 16 carbon atoms.

A. <u>The elongated molecules (C10-C16) are derived from:</u>

1. Palmitate: by cytoplasmic pathway.
2. fatty acids of diet.

B. The microsomal pathway needs malonyl CoA as acetyl donor and $NADPH+H^+$ as coenzyme.

C. <u>Function</u>: This system becomes active during myelination of nerves in order to provide C_{22} and C_{24} fatty acids which are present in sphingolipids.



Microsomal system for chain elongation.

Professor Said Orabi

IV. *Synthesis Of Unsaturated Fatty Acids:*

A. <u>Nonessential unsaturated fatty acids:</u>

1. These are fatty acids which contain one double bond e.g. palmitoleic acid (16:1) and oleic acid (18:1).
2. **Synthesis of oleic acid (oleyl CoA):** It is synthesized – in the microsomes - from stearyl CoA (active stearic acid).

B. <u>Essential fatty acid:</u> These are unsaturated fatty acids which contain more than one double bond.

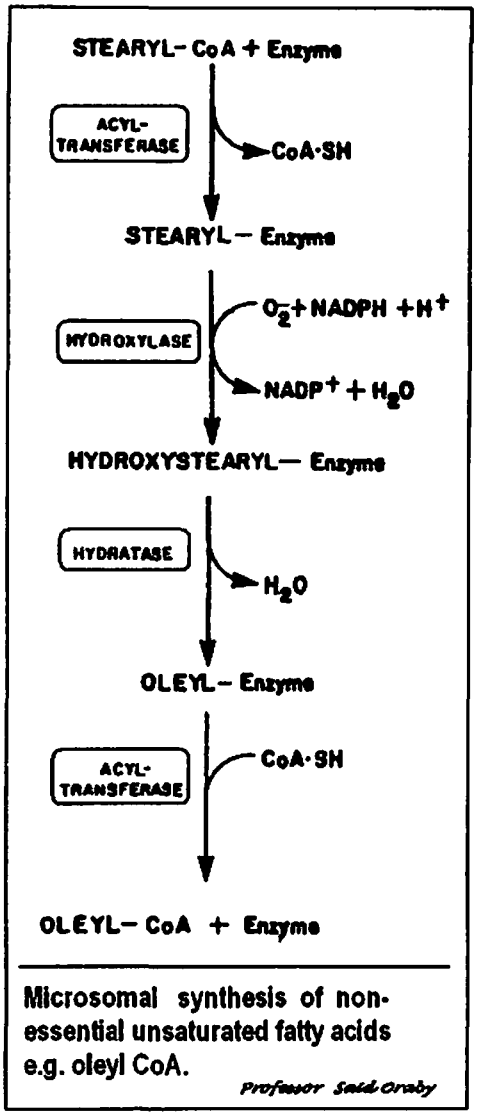
1. They are essential because they cannot be formed in the body and should be taken in the diet
2. **Examples:** linoleic acid (18:2) linolenic (18:3) and arachidonic acid (20:4).
3. **Sources:** Vegetable oils as corn oil and cotton seed oil.
4. **Functions:**

a- They are important for **normal growth.**

b- Synthesis of phospholipids: They enter in the structure of Phospholipids mainly in the 2nd position. Phospholipids have many functions as:

1) Enter in the structure of cell membranes.
2) They act as lipotropic factors i.e. prevent accumulation of fat in liver.
3) Dipalmityl lecithin acts as surfactant in lungs.
4) Cephalin is important for coagulation.

c- Prevention of atherosclerosis: Essential fatty acids combine with cholesterol forming esters which are rapidly metabolized by the liver. This prevents precipitation of free cholesterol along the endothelium of blood vessels ⇒ prevents atherosclerosis.



Microsomal synthesis of non-essential unsaturated fatty acids e.g. oleyl CoA.

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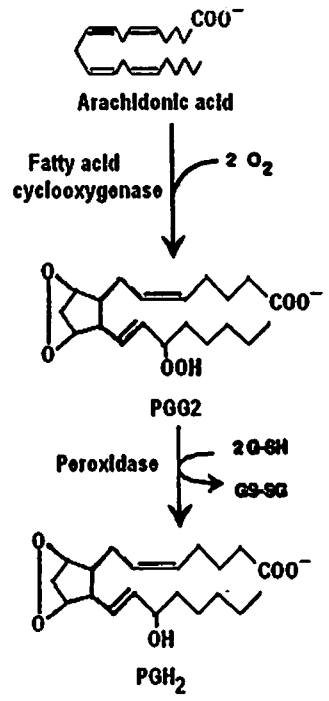
d- Synthesis of eicosanoids: Arachidonic acid gives rise to a group of compounds called **eicosanoids**. They comprise the prostanoids, leukotrienes (LTs) and lipoxins (LXs). Prostanoids include prostaglandins (PGs), prostcyclins (PGIs) and thromboxane TXs).

EICOSANOIDS

I. **<u>Definition:</u>** These are cyclic compounds that derived from arachidonic acid (eicosatetraenoic) (20 C) after cyclization of its carbons chain to form a ring.

II. Components of eicosanoids:

1. **Prostanoids:** which comprise prostaglandins, prostacyclins and thromboxanes. **Prostaglandins (PG) include many types** (A, B, D, E, F, H, G and I).
2. **Leukotriens (LT):**
   a) They are present in leucocytes, platelets and mast cells.



Oxidation and cyclization of arachidonic acid

III. *SYNTHESIS OF PROSTANOIDSS:*

A. The immediate precursor of prostanoids is ***arachidonic acid,*** which is 20 carbon polyunsaturated fatty acid that containing 4 double bonds (20:4 $\Delta^{5,8,11,14}$). Arachidonic acid is derived from **Phospholipids** present in all cell membranes by the action of phospholipase A_2 enzyme.

B. The first step in the synthesis of prostanoids is the oxidation and cyclization of arachidonic acid to give PGG_2 and PGH_2 (see the figure).

These reactions are catalyzed by two microsomal enzymes:

1. **Fatty acid cyclo-oxygenase**, which requires 2 oxygen molecules.
2. **Peroxidase,** which is dependant on reduced glutathione (G-SH).

C. PGH2 is the precursor all prostanoids.

D. Regulation of synthesis of prostanoids:

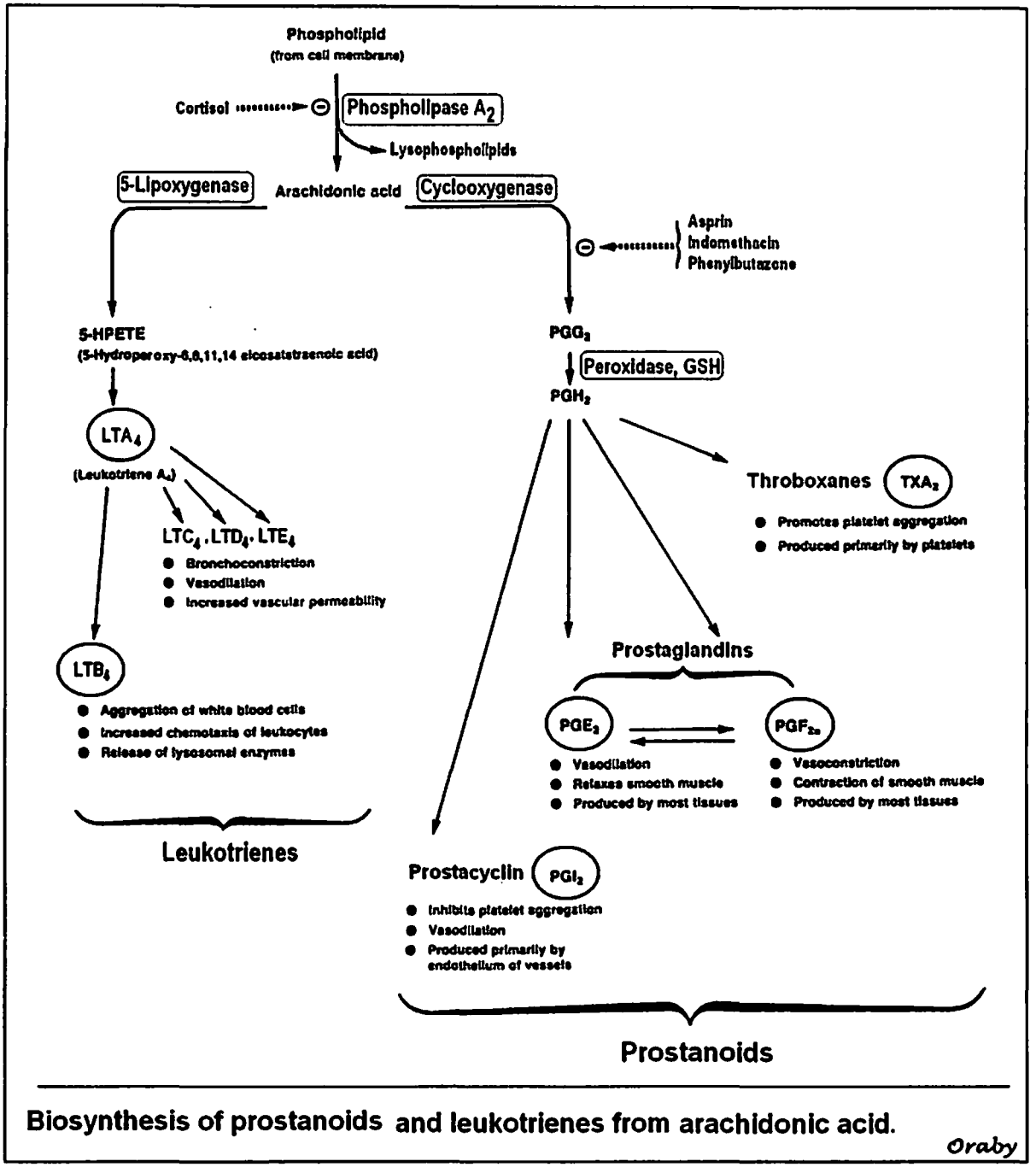
1. **Cortisol** inhibits the phospholipase A_2 activity,
2. **Aspirin,** indomethacin and phenylbutazone -which are antiinflamatory agents- inhibit cyclooxygenas. These agents do not affect synthesis of the leukotrienes.

IV. *SYNTHESIS OF LEUKOTRIENES:*

A. In neutrophils, arachidonic acid is converted to 5-hyroperoxy 6, 8, 11, 14 eicosatetraenoic acid (5-HPETE). This reaction is catalyzed by 5-lipooxygenase enzyme.

B. 5-HPETE is converted to a series of ***leukotrienes, (LT):*** LTA_4, LTB_4, LTC_4, LTD_4 and LTE_4.

C. In contrast to prostanoids synthesis, no drugs are known specifically to inhibit the lipoxygenase pathway.



Biosynthesis of prostanoids and leukotrienes from arachidonic acid.

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FUNCTIONS OF EICOSANOIDS:

A. ***Prostanoids:***

1. ***Thromboxanes:***
   - *a)* It is produced by platelets. It **stimulates platelets aggregations**.
2. ***Prostacyclin:***
   - *a)* It **inhibits platelets aggregations**.It is produced by endothelial cells of the blood vessels.
   - *b)* Vasodilatation.
3. ***Prostaglandins:***
   - *a)* **Prostaglandin E2 (PGE2):** Produced by most tissues.
     1) Vasodilatation.

2) Relaxesation of smooth muscles.

b) **Prostaglandin F2α (PGF2α):** Produced by most tissues.

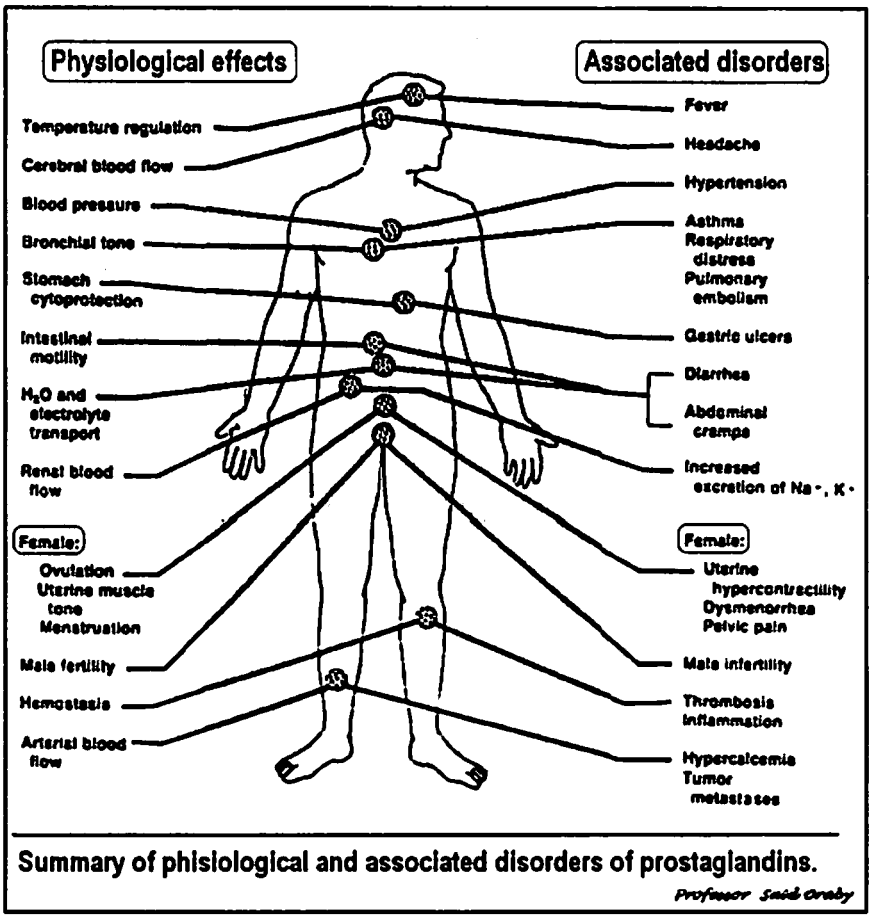
1) Vasoconstriction.

2) Contraction of smooth muscles.

> ***Note:*** Prostanoids have a **hormonal like action.** Prostanoidss differ from a true hormone in the following aspects:
>
> 1. They are formed in almost all tissues rather than in specialized glands.
> 2. They generally act locally rather than by circulating in the blood to the far target tissues.
> 3. Prostanoids are metabolized to inactive products at their site of synthesis and are not stored in any tissues.

B. ***Leukotrienes*** **(LTA_4):**

1. **Leukotrienes B_4 (LTB_4):**
   - *a)* **Movement and aggregation of white blood cells at the site of inflamation (chemotactic movement).**
   - *b)* **Release of lysosomal enzymes.**
2. **Leukotrienes (LTC_4, LTD_4, LTE_4):**
   - *a)* **Bronchoconstriction.**
   - *b)* **Vasodilatation.**
   - *c)* **Increased vascular permeability.**



Summary of phisiological and associated disorders of prostaglandins.

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C. In addition to the above functions, ecosanoids have other wide range of physiological functions as regulation of body temperature and controlling blood pressure. These functions are shown in the above figure.

D. Excess production of prostanoids results in group of symptoms including pain, inflammation, fever, nausea and vomiting (see figure).

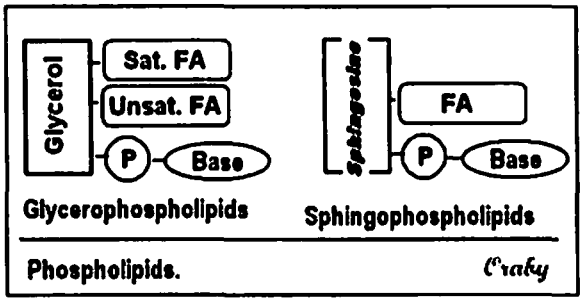
Metabolism of Conjugated Lipids

I. METABOLISM OF PHOSPHOLIPIDS:

A. Phospholipids are lipids containing phosphate.

B. They also contain alcohol (glycerol or sphingosine), fatty acid(s) and nitrogenous base.

C. The bases include; choline, serine, ethanolamine and inositol.



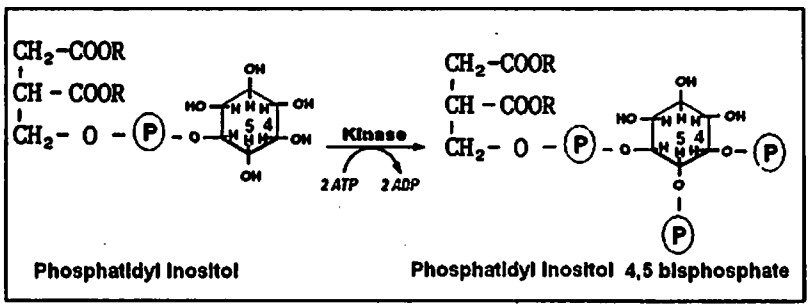
D. **Synthesis of lecithin (Phosphatidyl choline):** It is formed of glycerol, two fatty acids, phosphate and choline.

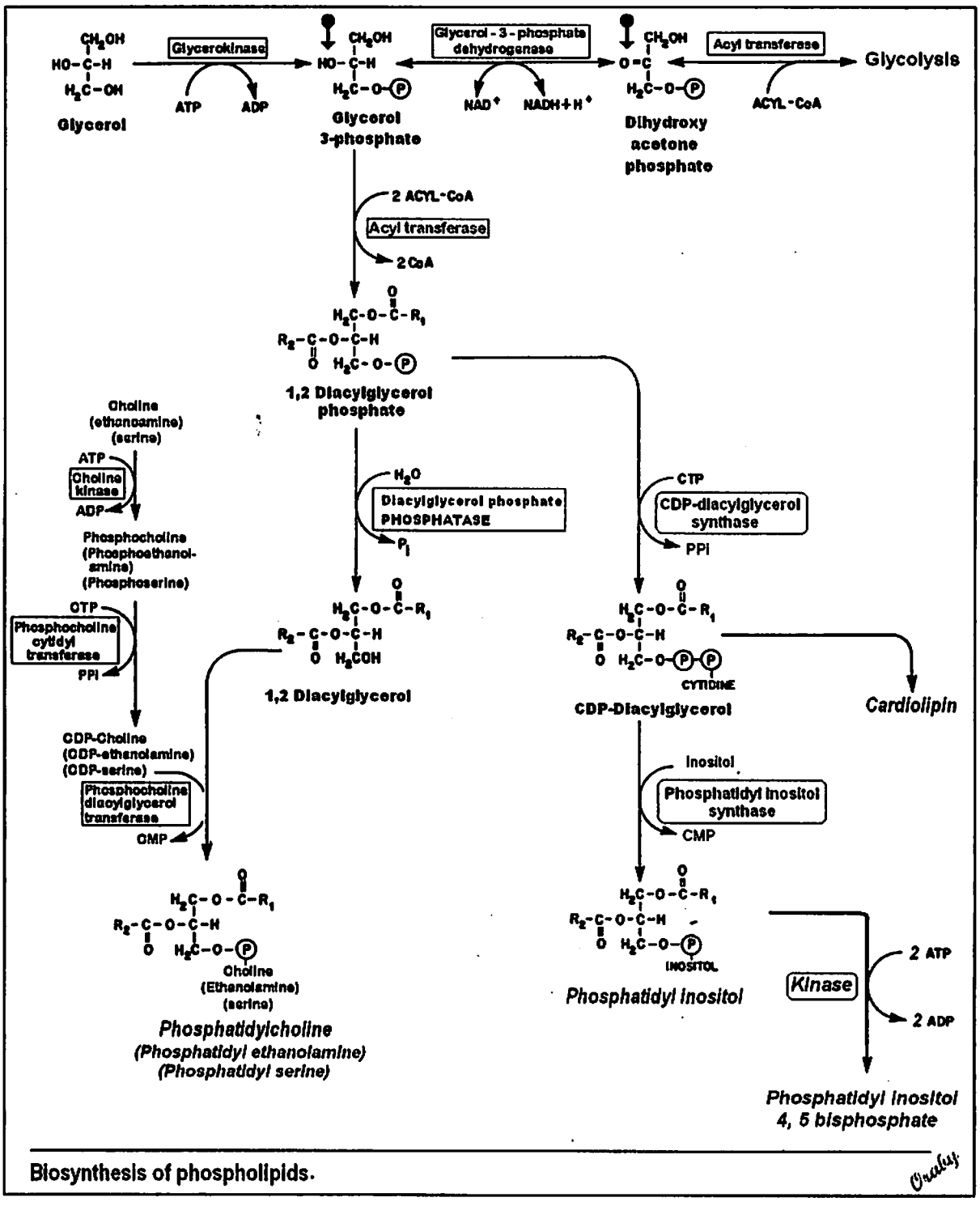
1. Activation of fatty acids into acyl CoA:
2. Synthesis of 1,2 diacylglycerol.
3. Activation of choline into CDP-choline.
4. Reaction of 1,2 diacylglycerol with CDP-choline to form lecithin.

E. **Synthesis of Phosphatidyl ethanolamine and phosphatidyl serine:** Same as lecithin but active ethanolamine (CDP-ethanolamine) or active serine (CDP-serine) are used instead of active choline.

F. **Synthesis of Phosphatidyl inositol and Phosphatidyl inositol 4,5 bisphosphate:**

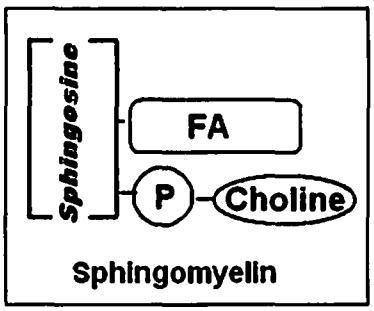
1. Activation of fatty acids into acyl CoA:
2. Synthesis of 1,2 diacylglycerol phosphate.
3. Reaction of 1,2 diacylglycerol phosphate with CTP to form CDP-diacylglycerol.
4. CDP-diacylglycerol reacts with inositol to form Phosphatidyl inositol.
5. Phosphatidyl inositol then accepts 2 phosphate from 2 ATP to form Phosphatidyl inositol 4, 5 bisphosphate.



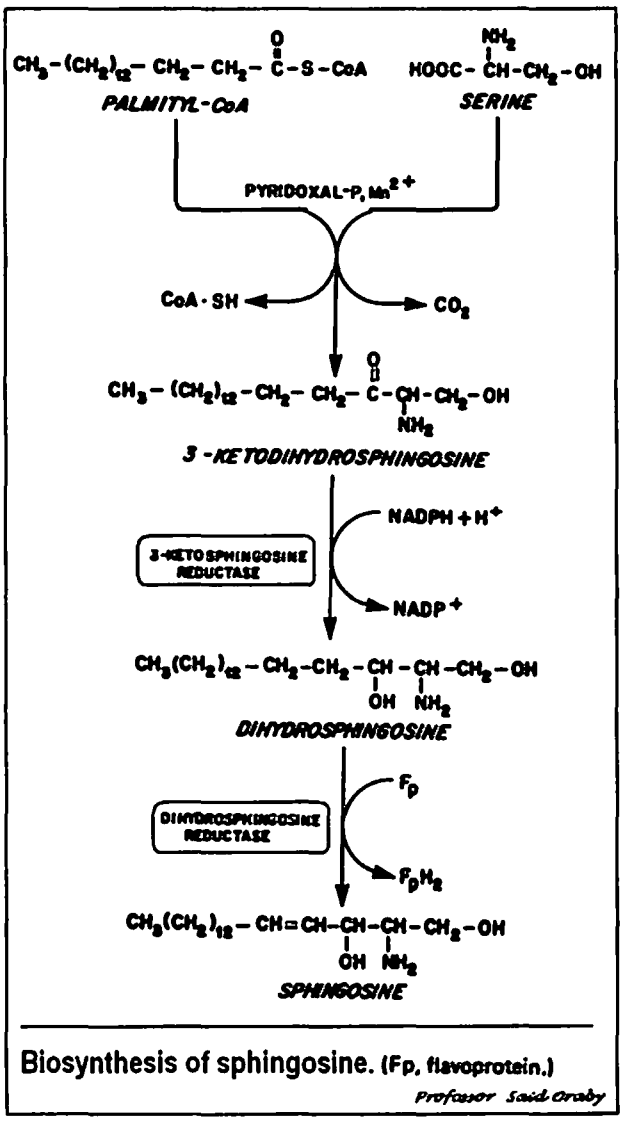


Biosynthesis of phospholipids.

G. **Synthesis of sphingomylin:** It is formed of: Sphingosine base, Fatty acyl CoA, phosphate and choline



1. **Synthesis of sphingosine:** It is formed of amino acid serine and active palmitic acid (16C): palmityl CoA ➔ sphingosine.
2. Then sphingosine reacts with acyl CoA to form ceramide.
3. Ceramide then reacts with CDP-choline to form sphingomyelin.



Biosynthesis of sphingosine. (Fp, flavoprotein.)

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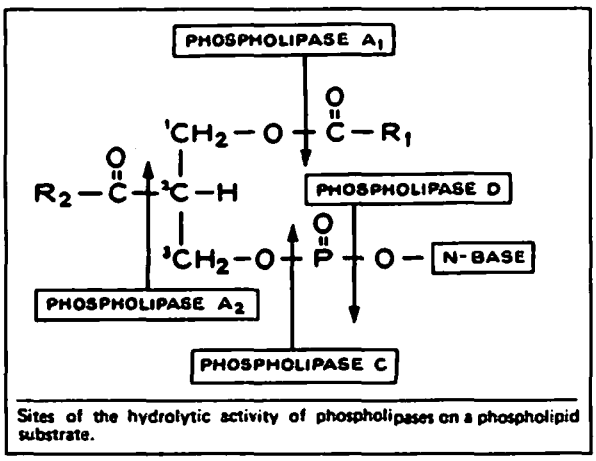
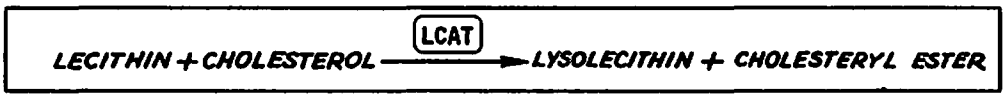


Sites of the hydrolytic activity of phospholipases on a phospholipid substrate.

H. **Degradation of phospholipids:**

1. Phosphoglycerides are degraded by phosphlipases A_1, A_2, C and D. They are present in most tissues and pancreatic juice.
2. Sphingomyelin is degraded by lysosomal enzymes, **sphingomyelinase. Its deficiency leads to a disease called** Niemann Pick disease. It is characterized by enlarged liver and spleen and death at early life.

3. A plasma enzyme called **LCAT** (lecithin cholesterol acyl transeferase) can act upon second fatty acyl CoA of lecithin (similar to phospholipase A2) converting it into lysolecithin.



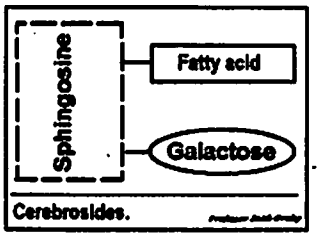
I. Functions of phospholipids:

1. Phospholipids enter in the **structure of cell membrane**.
2. Phospholipids containing choline (e.g. lecithin) act as **neurotransmitters**. They also act as **methyl donors** in transmethylation reaction.
3. Phospholipids act as **lipotropic factors** i.e. prevent accumulation of fat in liver and hence prevent fatty liver.
4. Dipalmityl lecithin acts as **surfactant** in the lungs. It prevents adherence of alveolar wall. Its deficiency leads to **respiratory distress** syndrome in premature babies.
5. Cephalin has a role in **coagulation** mechanism.
6. Lipositol (Phosphatidyl inositol) acts as precursor for **inositol triphosphate**. The latter acts as **2nd messenger**, mediating action of some hormones inside target cells.
7. Phospholipids in bile **make cholesterol soluble**. Their deficiency leads to cholesterol gallstones.

II. *METABOLISM OF GLYCOLIPIDS (SPHINGOLIPIDS):*

A. Glycolipids are lipids containing carbohydrates.

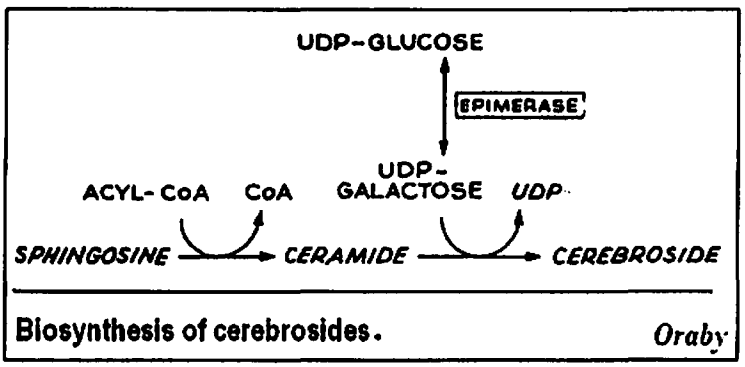
B. They also contain sphingosine and one fatty acid. They differ from each other according to the type of carbohydrate content.



C. **Synthesis of cerebrosides:** They Contain galactose.

1. Synthesis of sphingosine; See before.
2. Formation of ceramide.
3. Activation of galactose: ***UDP-Glucose → UDP Galactose.***

D. ***Degradation of cerebrosides*** is by ***glucocerebrosidase.*** Its defiency leads to a disease called **Gaucher's disease.** It is characterized by mental retardation and enlarged liver and spleen in children.



E. Lipidosis: Errors of phospholipids and sphingolipids metabolism:

These are a group of diseases in which there is an abnormal accumulation of phospholipids and glycolipids in nervous tissue. They are common in children. They are characterized by:

1. Deficiency of specific lysosomal enzymes responsible for degradation of sphingolipids.
2. Accumulation of sphingolipids in tissues leads to their enlargement e.g. liver and spleen enlargement.
3. Mental retardation is present in many diseases.
4. The most common diseases are **Gaucher's disease** and **Niemann Pick diseases (see the table below).**

Summary of the important lipidosis

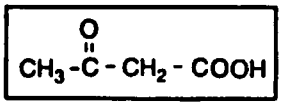
Disease	Enzyme Deficiency and Reaction Involved	Clinical Symptoms
Gaucher's disease	Glucocerebrosidase	Enlarged liver and spleen, mental retardation
Fabry's disease	Ceramide trihexosidase	Skin rashs, kidney failure ; full symptoms only in males (X-linked recessive)
Metachromatic leukodystrophy	Sulfatidase	Progressive nervous disorders due to demyelination; motor dysfunction
Krabbe's disease	Galactocerebrosidase	Severe mental retardation in infants; myelin almost absent
Niemann-Pick disease	Sphingomyelinase	Enlarged liver and spleen due to accumulation of sphingomyelin; fatal in early life
Tay-Sachs disease	Hexosaminidase	Mental retardation, blindness, demyelination, accumulation of Tay-Sachs ganglioside (G_{M2})
Generalized (G_{M1}) gangliosidosis	Galactosidase	Mental retardation, liver enlargement, skeletal deformation, accumulation of G_{M1} gangliosides

NOTE:
1- Defects in synthesis of phospholipids and sphingolipids ➔ Multiple sclerosis.
2- Defects in degradation of phospholipids and sphingolipids (lysosomal disorders) ➔ Sphingolipidosis.

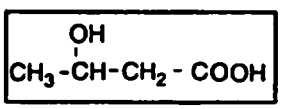
Acetone (Ketone) Bodies

I. ***DEFINITION:*** These are 3 compounds formed by the liver and include:

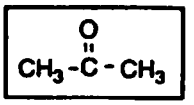
Acetoacetate



β-Hydroxybutyrate



Acetone



II. *FUNCTIONS (IMPORTANCE) OF KETONE BODIES:*

A. **Source of energy**: ketone bodies are used as a source of energy. They are converted into acetyl-CoA which is oxidized in tricarboxylic acid (TCA) cycle.

B. Skeletal muscles, cardiac muscles, kidneys and most of tissues can use ketone bodies as a source of energy in prolonged fasting and starvation.

C. **Brain tissue** can also oxidize ketone bodies within **5 to 6 days of starvation.** Note: brain never oxidizes fatty acids.

D. **Liver does not** contain enzymes for ketone bodies oxidation (ketolysis). Thus liver cannot oxidize them.

III. *SYNTHESIS OF KETONE BODIES (KETOGENESIS):*

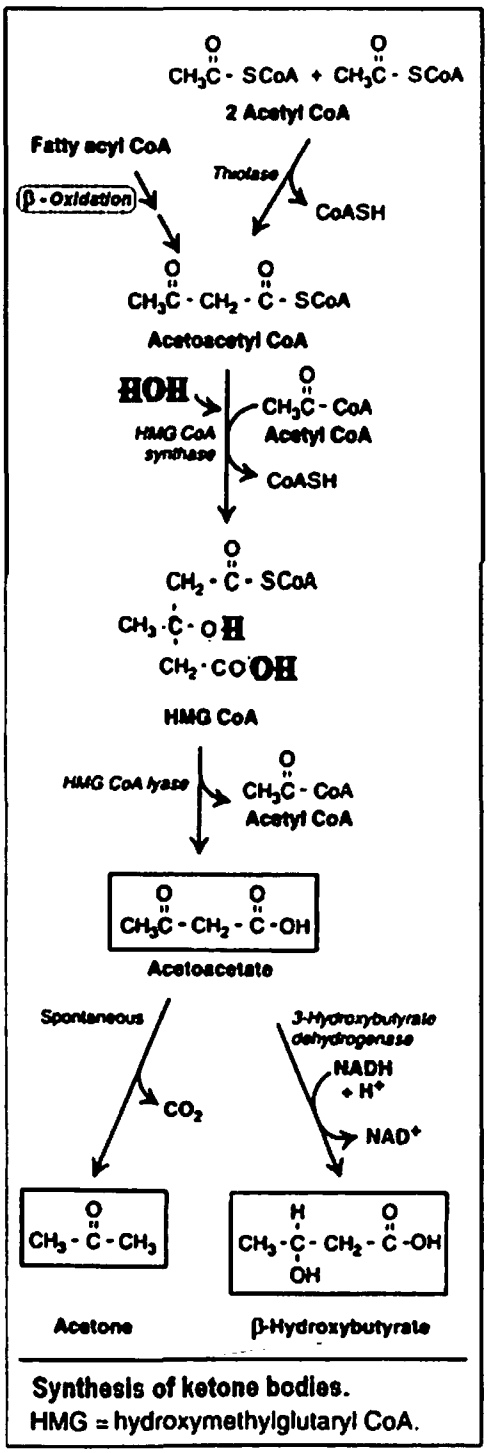
A. **Site:**

1. **Organ location:** Liver
2. **Intracellular location:** Mitochondria.

B. **Precursor:** Acetyl CoA (derived from fatty acids oxidation and ketogenic amino acids).

C. **Steps**: Acetoacetate is the first ketone body produced. Then both β-hydroxy butyrate and acetone are derived from it :

1. **Formation of acetoacetyl CoA:**
   a) From condensation of 2 acetyl CoA molecules.
   b) Acetoacetyl CoA may also be derived from β-oxidation of fatty acids (last 4 carbons).
2. **Formation of HMG-CoA (β-hydroxyl β-methyl glutaryl CoA):** By condensation of third molecule of acetyl CoA in the presence of HMG CoA synthase.
3. **Formation of acetoacetate** by HMG CoA lyase.



Synthesis of ketone bodies.
HMG = hydroxymethylglutaryl CoA.

4. **Formation of β- hydroxybutyrate and acetone:** Acetoacetate is either:
   a) Spontaneously decarboxylated into acetone.
   b) Reduced by hydroxybutyrate dehydroganase into β-hydroxy-butyrate.

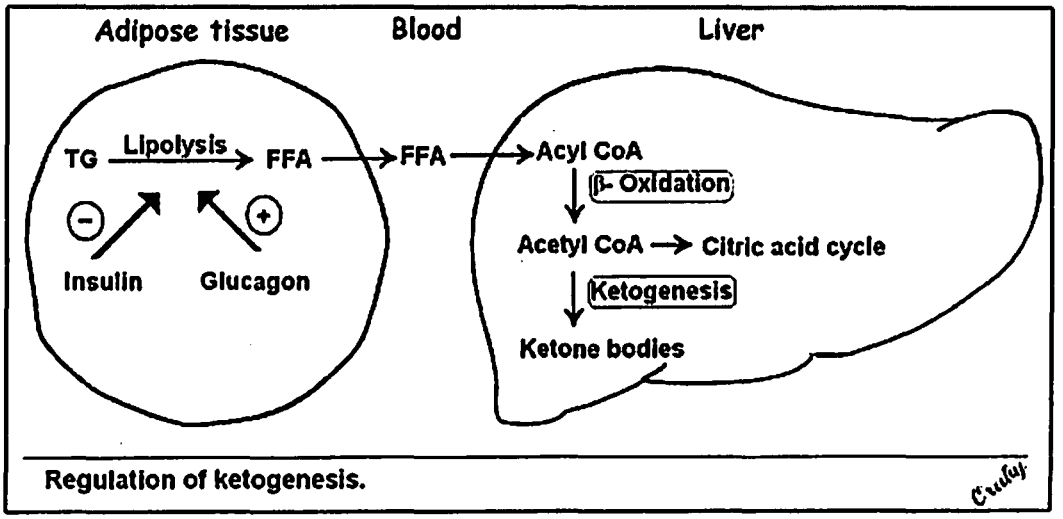
D. Conditions that increase ketogenesis:

1. Starvation.
2. Diabetes mellitus.
3. Low carbohydrate diet.
4. Hhypercatabolic states e.g. fevers, hyperthyroidism.

Note that both lipolysis and ketogenesis are activated by the same conditions.

E. Regulation of ketogenesis:

1. **After meal,** insulin is secreted, **and this inhibits lipolysis and in turn inhibits ketogenesis.**
2. **During fasting** anti-insulin hormones are secreted e.g. glucagon. **This stimulates lipolysis and in turn ketogenesis.**
3. **Explanation:**
   a) Conditions causing excessive lipolysis in adipose tissue leads to very high level of plasma free fatty acids (FFA).
   b) The liver in both fed and fasting states has the ability to **extract 30%** of the free fatty acids passing through it. Thus at high concentration of plasma FFA, the amount extracted by the liver is increased.
   c) In liver, FFA is activated to acyl CoA ➡ β-Oxidation to give acetyl CoA.
   d) Acetyl CoA is oxidized in citric acid cycle, but if the amount of acetyl CoA molecules is more than the capacity of CAC, they are used to form ketone bodies.



Regulation of ketogenesis.

IV. *OXIDATION OF KETONE BODIES (KETOLYSIS):*

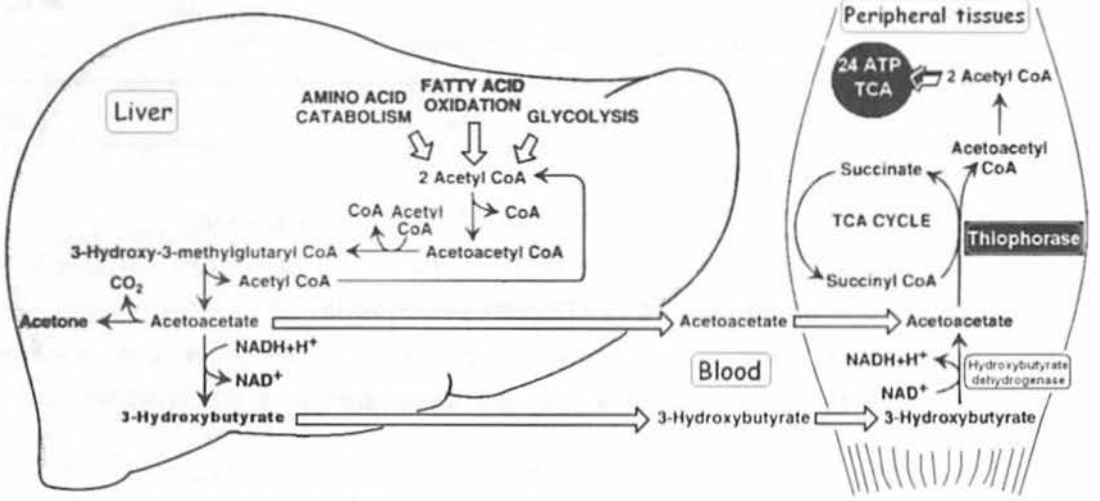
A. Site:

1. **Intracellular location:** Mitochondria.
2. **Organ location:** Extra hepatic tissues as muscles.

   Note: Ketolysis does not occur in liver because it does not contain enzymes for it.

B. Steps:

1. Acetone is volatile and removed in the expired air.
2. β-Hydroxybutyrate is converted into acetoacetate by hydroxybutyrate dehydrogenase enzyme.
3. Acetoacetate is then activated into acetoacetyl CoA by:
   a) **Thiophorase enzyme** in the presence of succinyl CoA.
   b) Acetoacetyl CoA synthetase enzyme, in the presence of ATP.
4. Acetoacetyl CoA is split into two molecules of acetyl CoA which are oxidized in citric acid cycle. Each gives 12 ATP.



Ketone bodies synthesis in the liver [KETOGENESIS] and utilization in peripheral tissues [KETOLYSIS].

V. *BLOOD KETONE BODIES AND KETOSIS:*

A. Blood ketone bodies concentration is less than 3 mg/dl.

B. Ketonemia: is the increase of blood ketone bodies above normal concentration.

1. It occurs when the rate of formation of ketone bodies (ketogenesis) is grater than the rate of their oxidation (ketolysis).
2. If the condition is severe, ketonemia may lead to acidosis (**ketosis**).

C. Urine ketone bodies: is less than 40 mg/day.

D. Ketonuria: is the increase of urine ketone bodies concentration above normal concentration:

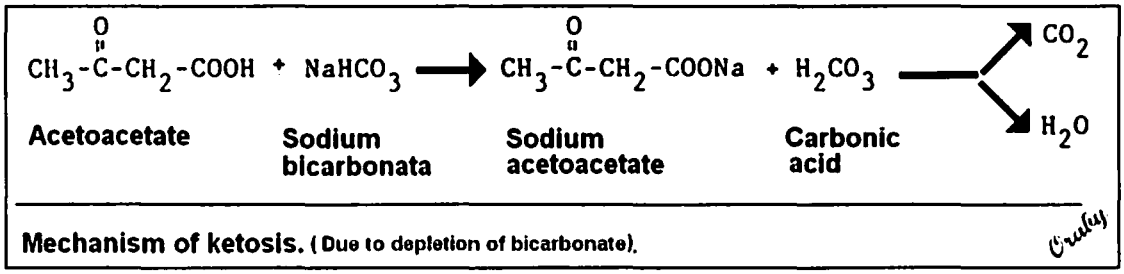
1. It usually occurs with ketonemia.

E. Causes of ketonemia and ketonuria:

1. Starvation
2. Severe diabetes mellitus.
3. Hypercatabolic states e.g. diarrhea and fever.

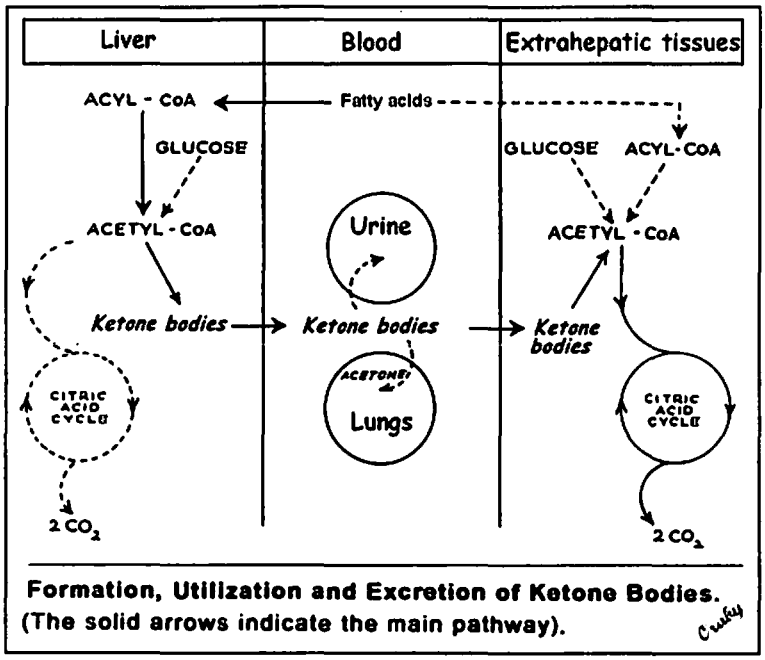
F. Ketosis (= ketoacidosis):

1. **Definition**: It is a condition of **metabolic acidosis** results from ketonemia.
2. **Mechanism:**
   a) An increase of ketone bodies in the blood is neutralized by blood buffers mainly bicarbonate (HCO_3^-).
   b) Bicarbonate will be depleted and this leads to decreased blood pH (acidosis).



Mechanism of ketosis. (Due to depletion of bicarbonate).

3. **Effects of acidosis:**
   a) Acidosis causes dizziness, loss of concentration...etc.
   b) Acidosis causes transfer of potassium (K^+) ions from intracellular fluid to blood leading to **($\uparrow K^+$) hyperkalemia.**
   c) **Ketotic coma:** In severe cases of ketosis as in uncontrolled diabetes mellitus, coma may be developed and the condition may be fatal.



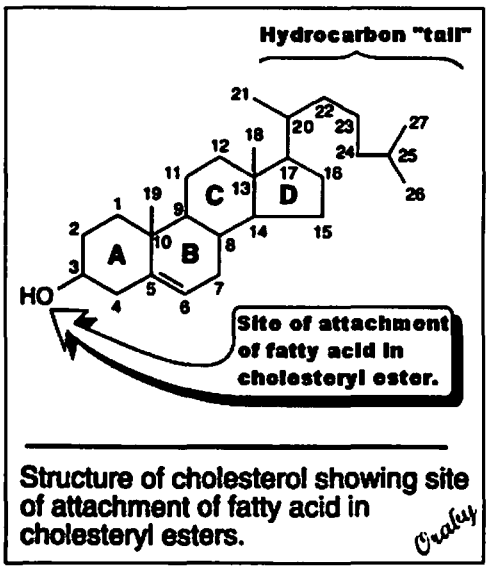
Formation, Utilization and Excretion of Ketone Bodies.
(The solid arrows indicate the main pathway).

Metabolism of Cholesterol

I. STRUCTURE:

A. Cholesterol is an animal sterol.

B. It is a solid alcohol having –OH group at C_3.



Structure of cholesterol showing site of attachment of fatty acid in cholesteryl esters.

II. SOURCES OF CHOLESTEROL:

A. **Endogenous**: Cholesterol is formed in the body almost in all nucleated cells from Acetyl-CoA (about 700 mg/day).

B. **Exogenous**: Cholesterol occurs only in food of animal origin such as egg yolk, meat, liver and brain. Diet supplies about 400 mg/day.

III. SYNTHESIS OF CHOLESTEROL:

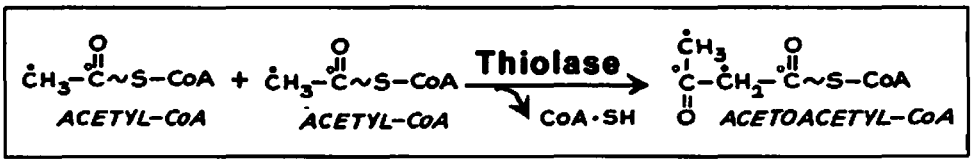
A. **Location**:

1. **Intracellular location**: Cytosol.
2. **Organ location**:
   a) **Liver** is the major site of cholesterol synthesis.
   b) Other tissues e.g. intestine, adrenal cortex, gonads and skin.

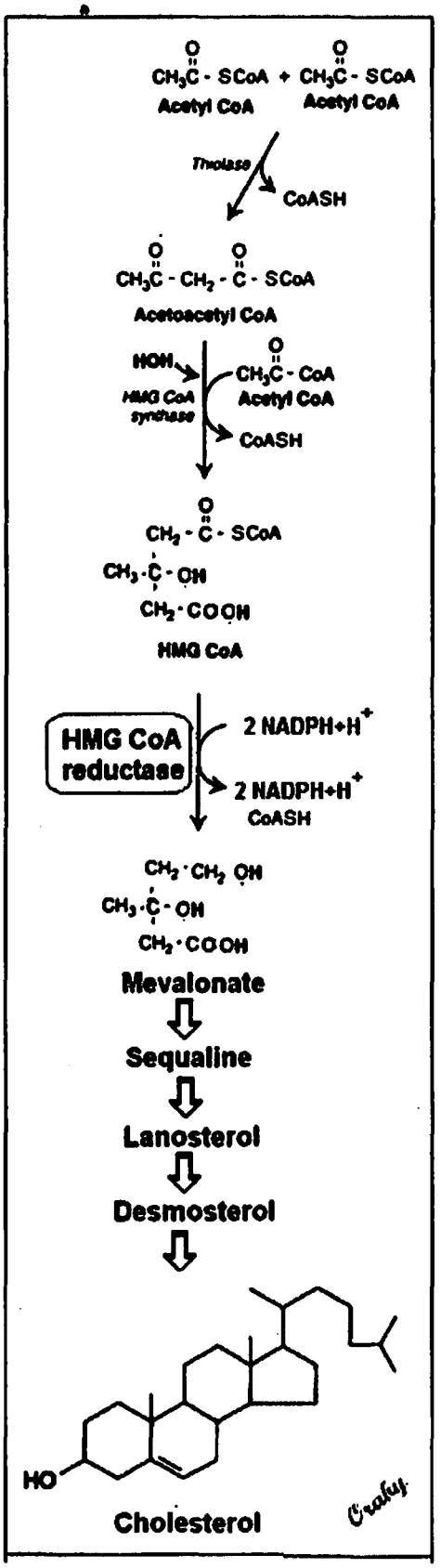
B. **Precursor**: Acetyl-CoA.

C. **Steps**:

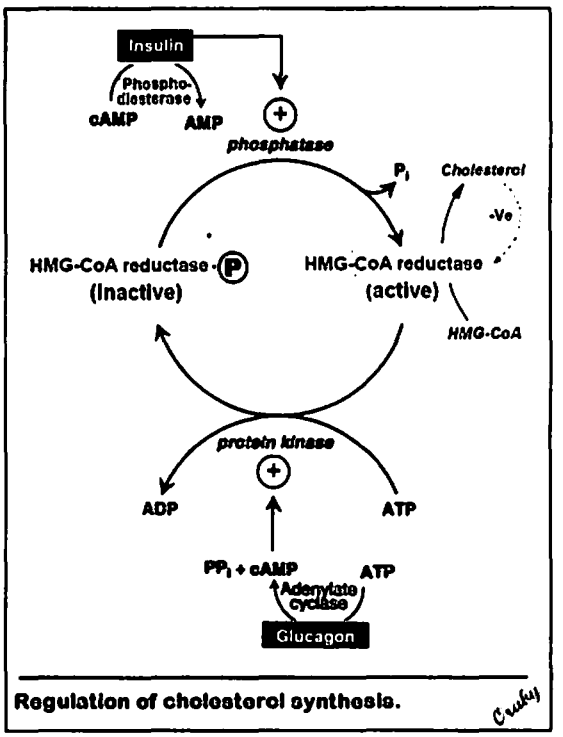
1. **Formation of acetoacetyl CoA**: by condensation of two molecules of acetyl CoA:



2. Conversion of acetoacetyl CoA to cholesterol.

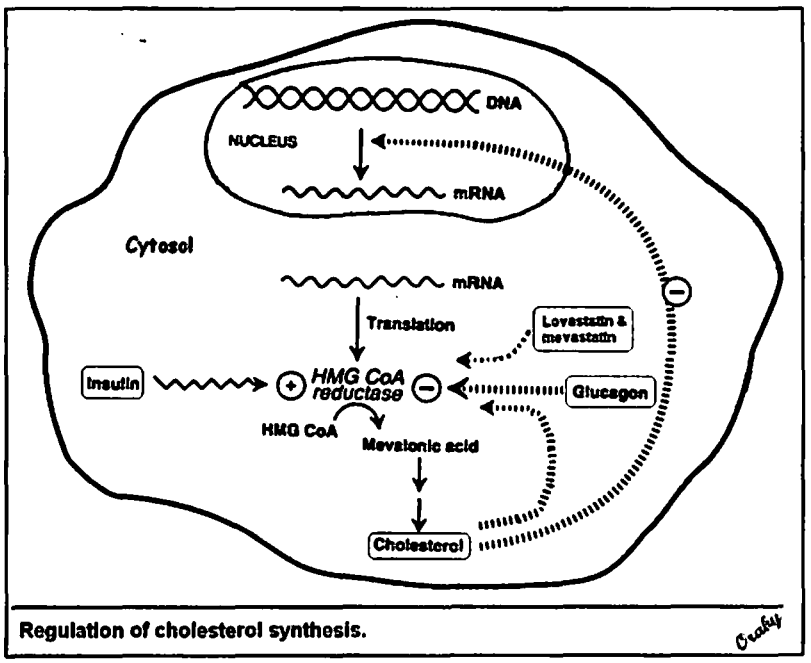


D. <u>Regulation of cholesterol synthesis</u>: ***HMG CoA reductase*** is the key enzyme for cholesterol synthesis. It is present in two forms: active dephosphorylated and inactive phosphorylated. It is Regulated through:



Regulation of cholesterol synthesis.

1. **Feed back Inhibition:** Cholesterol *(the end product of the pathway)* acts as feed back inhibitor of HMG CoA reductase enzyme. Thus, it decreases more cholesterol synthesis.
2. **Feed back regulation:** Cholesterol *(either synthesized by the cell or reaching it from diet)* inhibits HMG CoA reductase **<u>gene</u>**. This decreases transcription and synthesis of HMG CoA reductase.
3. **Hormonal regulation:**
   a) **Glucagon:** Inhibits HMG CoA reductase (through stimulation of cAMP and protein kinase).
   b) **Insulin:** Stimulates HMG CoA reductase (through stimulation of phosphodiesterase and phosphatase enzymes).
4. **Inhibition by drugs:** Lovastatin and mevastatin are drugs, which inhibit HMG CoA reductase by reversible competitive inhibition. They are used to decrease plasma cholesterol levels in patients with hyper-cholesterolemia.



Regulation of cholesterol synthesis.

IV. *FUNCTIONS OF CHOLESTEROL:*

A. Cholesterol enters in the structure of **every body cell** (e.g. cell membrane).

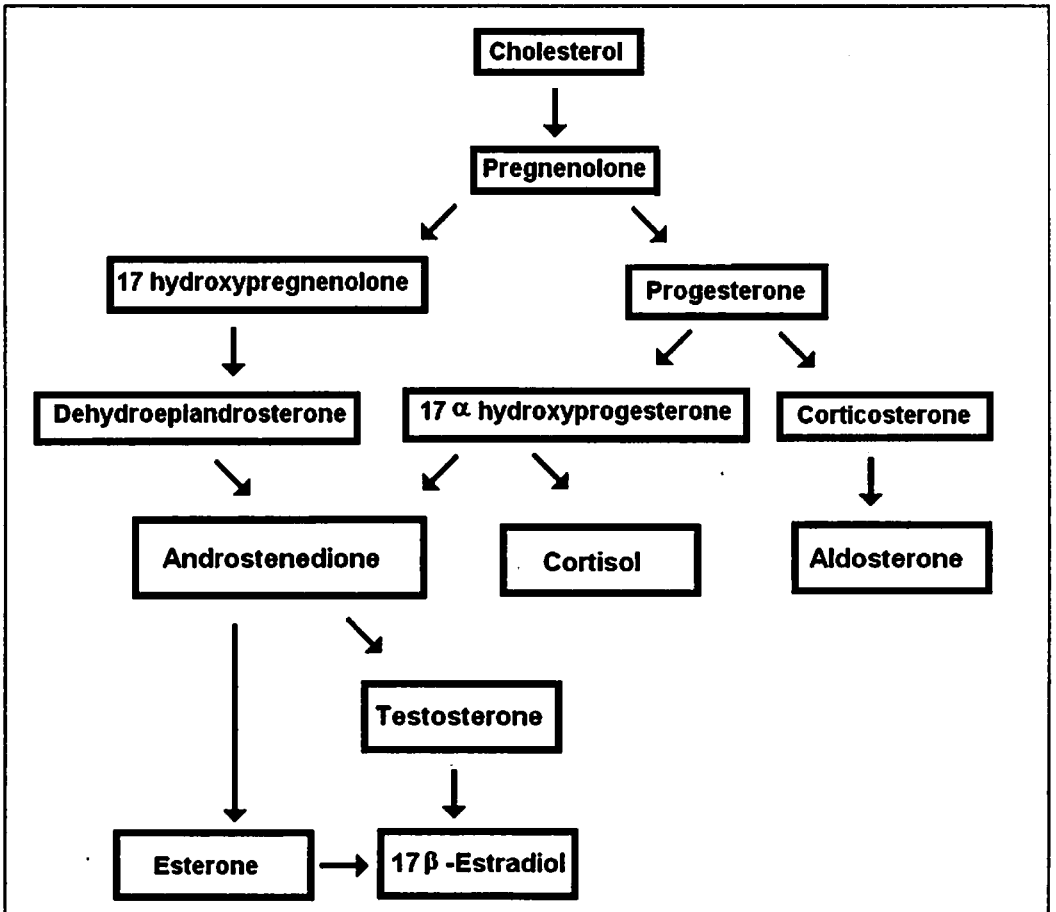
B. **Cholesterol is the precursor of:**

1. **Vitamin D_3:** (subcutaneous fat) ➔ 7-dehydrocholesterol ➔ Vitamin D_3.
2. **Steroid hormones:**
   a) **Estrogens and progesterone** (ovaries).
   b) **Testosterone and androgens** (testes).
   c) **Glucocorticoids and mineralocorticoids** (Adrenal cortex).
3. **bile acids: (Liver)**

1. **Synthesis of vitamin D_3:** See part I, vitamins.

Cholesterol ➔ 7-Dehydrocholesterol ➔ Vitamin D3 ***(SKIN)*** ➔ 25 hydroxy D3 ***(LIVER)*** ➔ 1,25 dihydroxy D3 ***(KIDNEY).***

2. **Synthesis of steroid hormones** (androgens, estrogens, progesterone and corticoids):

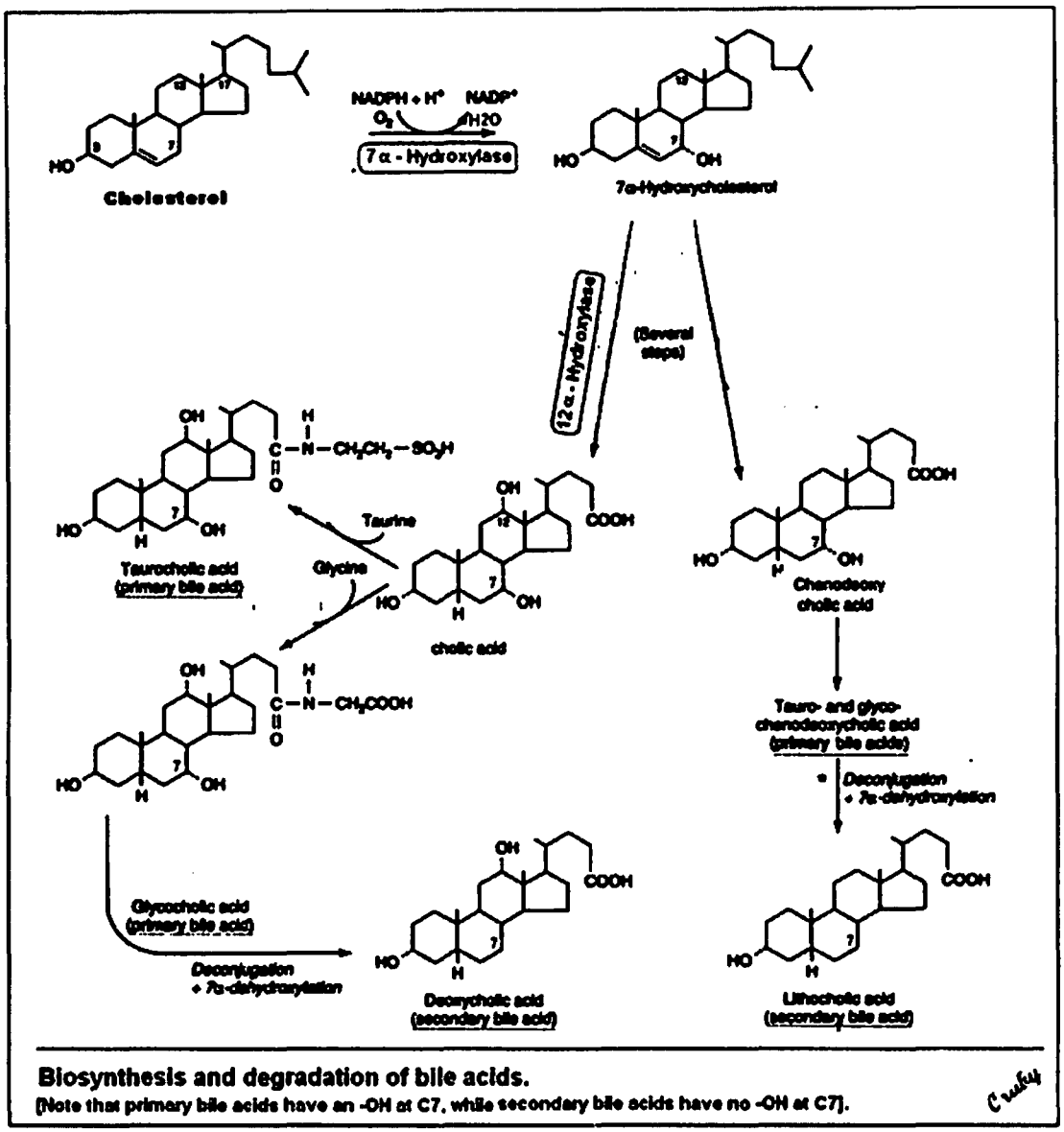


The biosynthesis of steroid hormones from cholesterol. Oraby

3. Synthesis of bile acids and salts:

a) Primary bile acids are cholic and chenodeoxy cholic acid.

b) Secondary bile acids are deoxycholic acid and lithocholic acid.



Biosynthesis and degradation of bile acids.
(Note that primary bile acids have an -OH at C7, while secondary bile acids have no -OH at C7).

V. *EXCRETION OF CHOLESTEROL:* About **one gram** of cholesterol is excreted **daily.** It is secreted as cholesterol, bile acids and coprostanol:

A. ½ Gram cholesterol is excreted as such with bile, which transports it to the intestine for elimination.

B. ½ Gram cholesterol is converted to bile acids, which is excreted in the feces.

C. Some cholesterol is synthesized by intestinal cells and modified by bacteria before excretion. Bacterial enzymes reduce cholesterol into **coprostanol**, which is excreted into feces.

VI. *PLASMA CHOLESTEROL :*

A. Cholesterol present in plasma is either free or esterified (cholesteryl ester).
   1. Total plasma cholesterol: 140 –220 mg/dl.
   2. Free plasma cholesterol: 26 – 126 mg/dl.

B. **Hypercholesterolemia:**
   1. **Definition:** It is increased plasma cholesterol concentration above 220 mg/dl.
   2. **Causes:**
      a) **Diet** rich in carbohydrate, cholesterol and saturated fatty acids.
      b) **Hypothyroidism** as thyroxin stimulates conversion of cholesterol to bile acids.
      c) **Diabetes mellitus.**
      d) **Kidney affection** (nephrotic syndrome) unknown mechanism.
      e) **Obesity.**
      f) **Obstructive jaundice** due to decreased excretion of cholesterol and bile acids.
      g) **Familial hypercholesterolemia.**

C. **Hypocholesterolemia:**
   1. **Definition:** It is decreased plasma cholesterol concentration below 140 mg/dl
   2. **Causes :**
      a) **Prolonged fasting** which causes decreased secretion of insulin (decreased activation of HMG-CoA reductase).
      b) **Diet** rich in unsaturated fatty acids and poor in saturated fatty acids, carbohydrate and cholesterol.
      c) **Liver diseases,** as liver is the site where most plasma cholesterol is synthesized.
      d) **Hyperthyroidism.**
      e) **Chronic infection** as tuberculosis.

VI. *TRANSPORT OF CHOLESTEROL:*

A. Cholesterol is hydrophobic. It is transported in plasma in the more soluble lipoprotein forms: LDL, VLDL and HDL (see plasma lipoproteins).

B. Free cholesterol is removed from tissues by HDL and transported to be excreted by the liver.

C. Cholesterol ester is the storage form of cholesterol: It is formed in both tissues and plasma.

1. In tissues (liver), cholesterol is esterified by **ACAT enzyme** (acyl CoA cholesterol acyl transferase):

Cholesterol + Acyl CoA ➔ Cholesteryl ester + CoASH

2. In plasma, cholesterol is esterified by **LCAT enzyme** (lecithin cholesterol acyl transferase). LCAT is associated with HDL.

Cholesterol + lecithin ➔ Cholesteryl ester + lysolecithin

PLASMA LIPIDS AND PLASMA LIPOPROTEINS

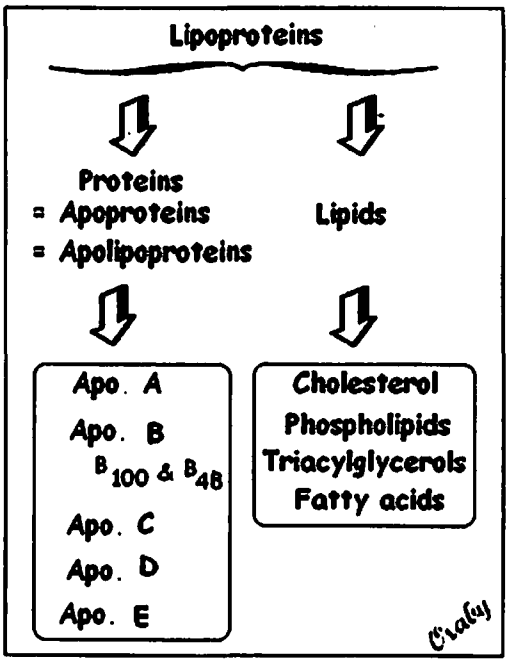
I. ***PLASMA LIPIDS:*** During fasting, total plasma lipids concentration ranges from 360-820 mg/dl. They include:

1-Cholesterol :	**140-220 mg/dl** **70% Cholesteryl esters** **30% Free cholesterol**
2-Phospholipids :	150-200 mg/dl
3-Triacylglycerols :	40-160 mg/dl
4-Free fatty acids :	6-16 mg/dl

II. *PLASMA LIPOPROTEINS :*

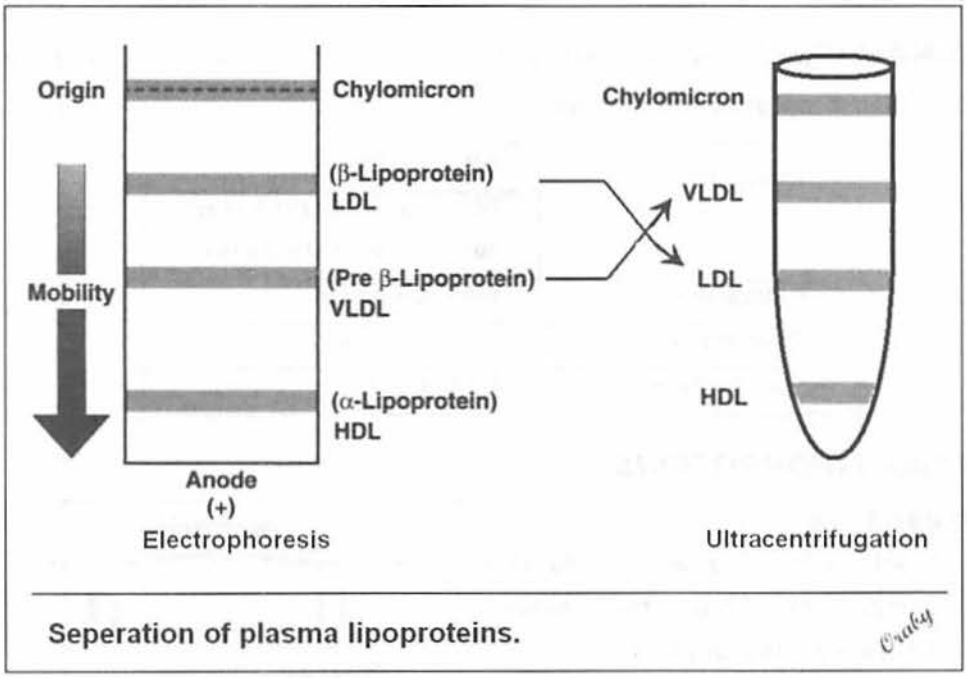
A. Importance:

1. Lipids alone are water insoluble compounds. Thus, they cannot be transported in plasma.
2. Lipids are conjugated to proteins to form lipoproteins, which are water-soluble and can be transported in plasma.
3. These proteins are synthesized by the liver and called apolipoproteins (or apoproteins). They are five classes: A, B, C, D and E.
4. Failure of liver to synthesize apolipoproteins leads to accumulation of fat in liver and this condition is called **fatty liver** (see later).



B. Methods of separation of plasma lipoproteins:

1. **Ultracentrifugation:**
   a) Ultracentrifugation means centrifugation of compounds at high speed ~ 40,000 rounds per minutes (RPM).
   b) By ultracentrifugation, plasma lipoproteins are separated into five fractions according to their density. These are chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL) and albumin free fatty acids (FFA) complex.
2. **Electrophoresis :**
   a) By electrophoresis, plasma lipoproteins are separated into five fractions. These are chylomicrons, β-lipoprotein, pre β-lipoprotein, α-lipoprotein and albumin-FFA complex.



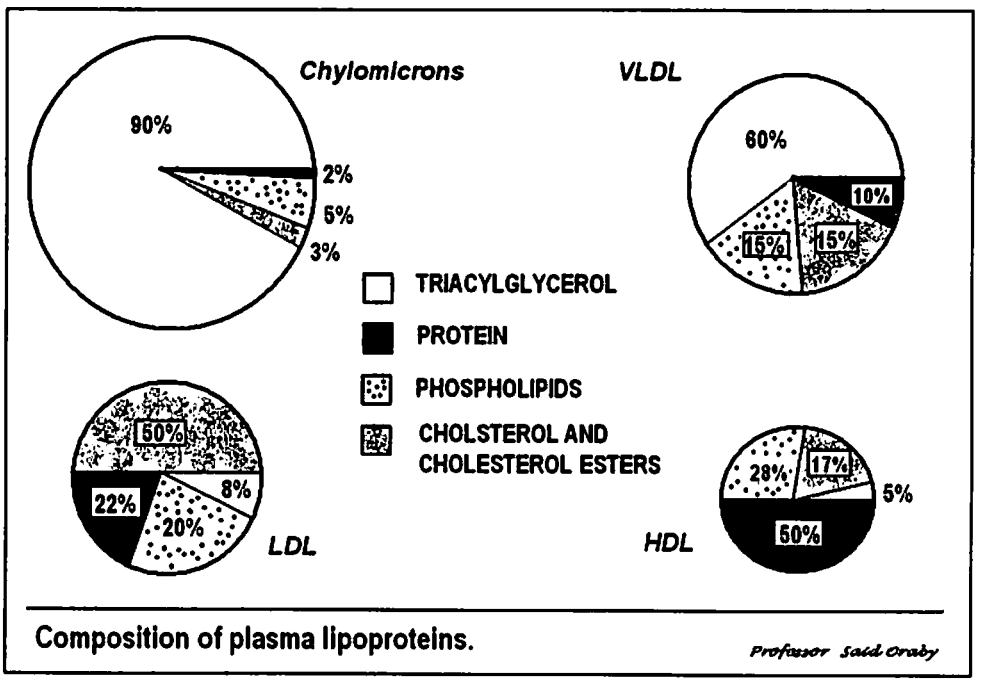
Seperation of plasma lipoproteins.

C. Fractions of plasma lipoproteins:

Each fraction of the five plasma lipoproteins (chylomicrons, VLDL, LDL, HDL and albumin free fatty acids complex) contains almost all types of lipids i.e. triacylglycerols, phospholipids, cholesterol, and free fatty acids. They differ in:

1. The main lipid content of each fraction.
2. Source of each fraction.
3. Types and amounts of associated proteins (apolipoproteins). The protein associated with fatty acids is albumin. The proteins associated with other types of lipids are globulins.

FRACTION	SOURCE	MAIN LIPID	APOLIPOPROTEINS	
			Amount	Types
Chylomicrons	Intestine	TG	2%	A, B_{48}, C &E.
VLDL	Liver	TG	10%	B_{100}, C & E.
LDL	Blood from chylomicrons and VLDL	Cholesterol, cholesteryl esters and phospholipids	22%	B_{100}
HDL	Liver	Cholesterol, cholesteryl esters and phospholipids	50%	A, C, D &E.
FFA-Albumin	Adipose tissue	FFA	99%	Albumin



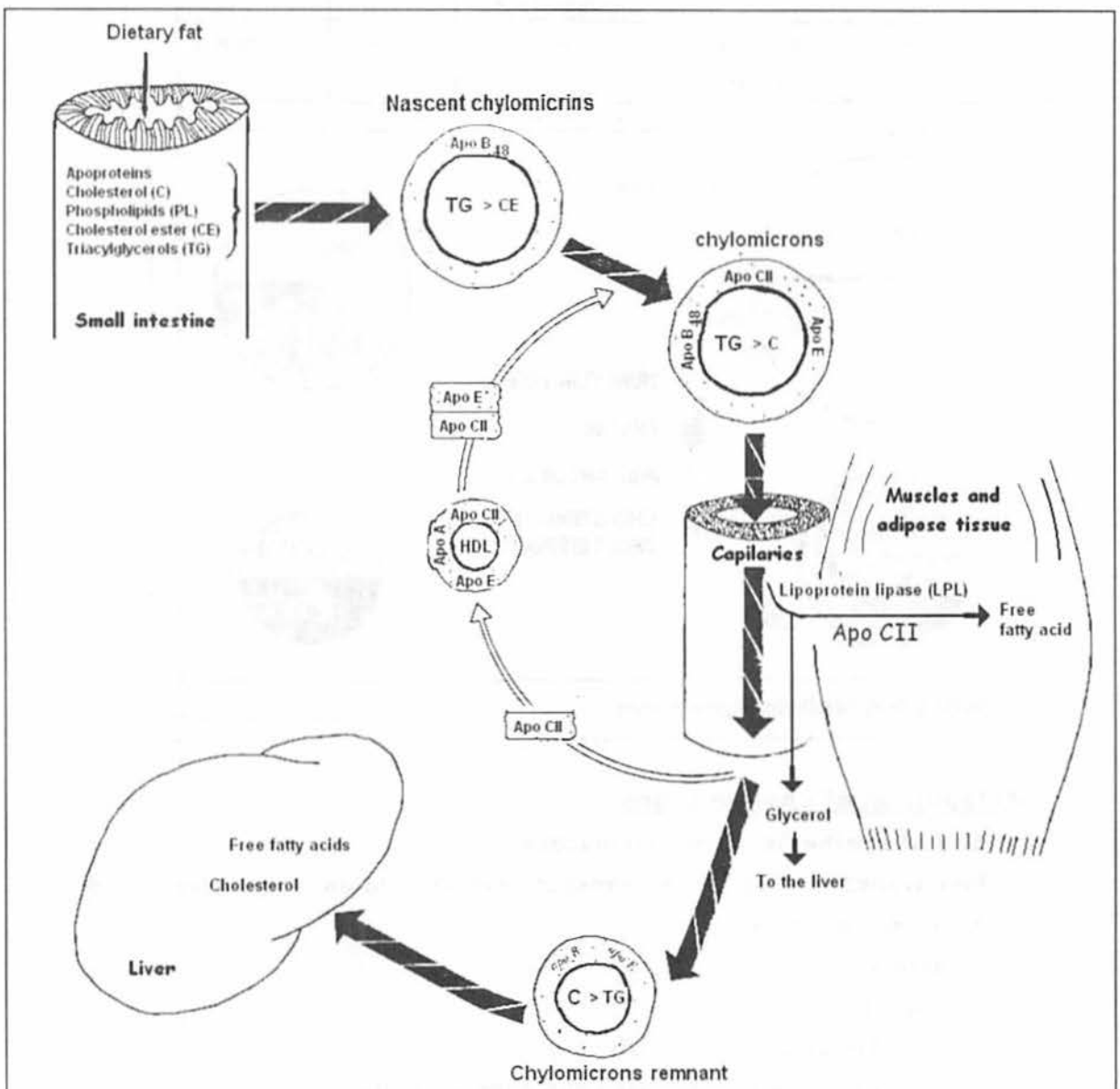
Composition of plasma lipoproteins.

Professor Said Orabi

D. Metabolism of chylomicrons:

1. **Site of synthesis:** Intestinal mucosa.
2. **Functions:** Chylomicrons transport **dietary lipids** from intestine ➔ Blood ➔ Peripheral tissues.
3. **Structure:**
   a) **Lipids:**
      1) Triacylglycerols, TG (main component).
      2) Cholesterol ester (CE) and phospholipids.
      3) Fat soluble vitamins.
   b) **Proteins:** 2% and include apo B_{48}, apo E and apo C (CII).

4. **Catabolism:**
   - *a)* The particles released by intestinal mucosal cells are called "nascent" chylomicrons and contains apolipoprotein B_{48}.
   - *b)* When it reaches the plasma, it receives -from circulating HDL- two apolipoproteins E and C and converted into chylomicrons.
   - *c)* The triacylglycerols component of chylomicrons will be hydrolyzed into glycerol and fatty acids. This reaction is catalyzed by lipoprotein lipase enzyme, which is activated by apo CII.
   - *d)* After hydrolysis of TG, the chylomicron particles begin to shrink. In addition, the apo CII returns to HDL. The remaining particles are called chylomicron remnants.



Metabolism of chylomicrons.

Oraby

e) Chylomicron remnants are removed from circulation by the liver. Hepatocyte membranes contain receptors that recognize the both apo E and apo B_{48}.

f) The cholesterol released from chylomicron remnants in liver appears to regulate the rate of cholesterol synthesis by inhibiting HMG CoA reductase.

Summary of chylomicrons metabolism:
1-Site of synthesis: intestinal mucosal cells
2-Functions: transport dietary lipids from intestine to peripheral tissues.
3-Structure:
a-Main lipids: triacylglycerols. Chylomicrons contains also cholesterol, phospholipids and fat soluble vitamins.
b-Proteins: (2%), apo B_{48} and receives apo CII and apo E from HDL.
4-catabolism: TG are hydrolyzed by lipoprotein lipase (which is activated by apo CII). The remaining parts are chylomicron remnants, which are then taken up by the liver. Hepatocyte receptors can recognize apoB_{48} and apo E.

5. **Disorders of chylomicrons metabolism:**
   a) Deficiency of lipoprotein lipase: Leads to **hyperlipoproteinemia**.
   b) The disease is called **familial lipoprotein lipase deficiency**; it is characterized by marked increase of plasma chylomicrons, especially after fatty meal.

E. Metabolism of very low density lipoproteins (VLDL):

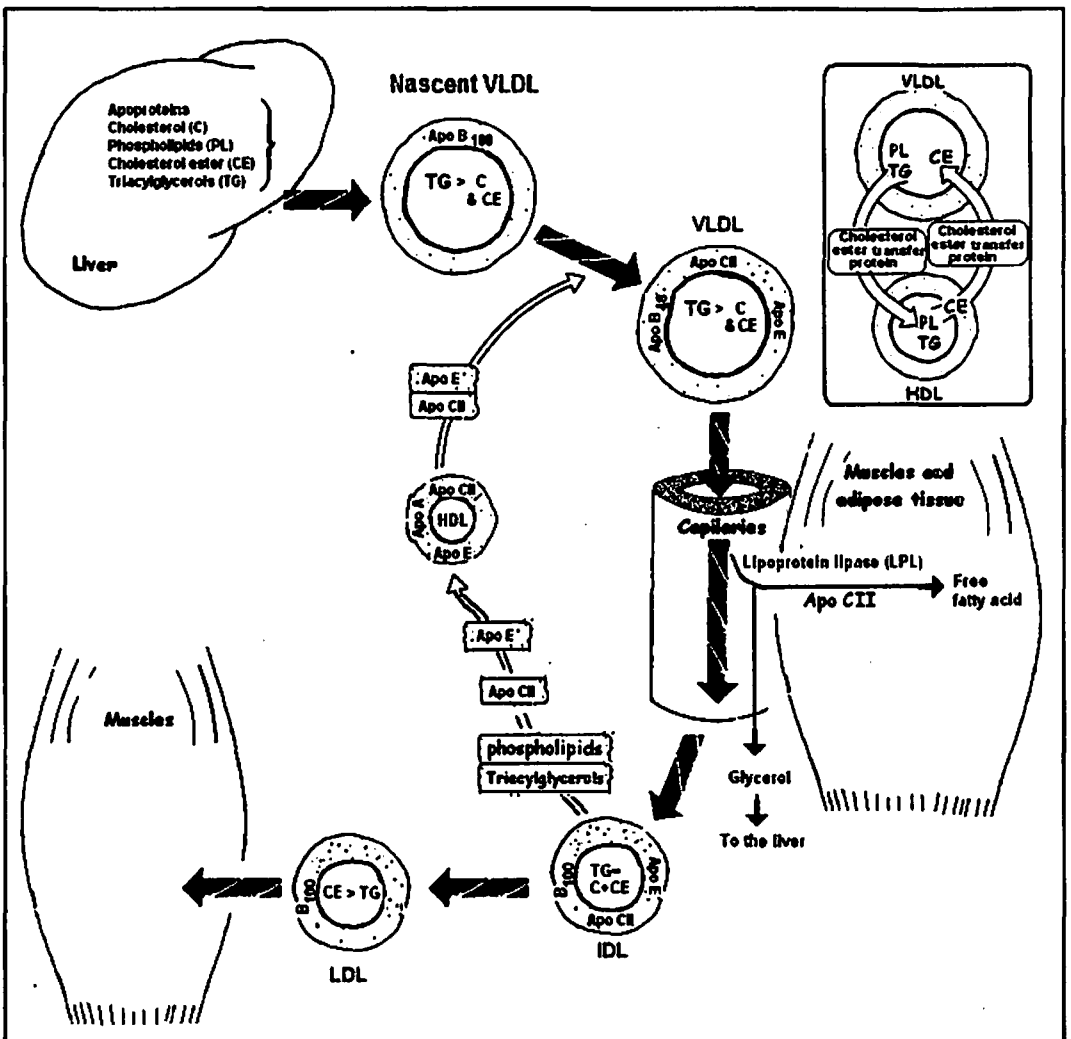
1. **Site of synthesis:** Liver
2. **Functions**: VLDL carries lipids from liver to the blood to the peripheral tissues.
3. **Structure:**
   a) **Lipids:**
      1) Triacylglycerols, TG (main component).
      2) Cholesterol esters and phospholipids.
   b) **Proteins:** 10% and include : apo B_{100}, apo E and apo C (CII)
4. **Catabolism:**
   a) The particles released from the liver are called **nascent VLDL** and contain B_{100}.
   b) When it reaches the plasma, it receives- from circulating HDL- two apolipoproteins; E and C and converted into VLDL.
   c) The triacylglycerols component of VLDL will be hydrolyzed into glycerol and fatty acids. This reaction is catalyzed by lipoprotein lipase enzyme, which is activated by apo CII.
   d) After hydrolysis of TG, the particles begin to shrink. The remaining particles are called **intermediate density lipoproteins (IDL)**.

e) IDL is converted into LDL by transferring phospholipids, apo CII, and apo E to HDL.

f) LDL are removed from circulation by tissues as muscles (discussed later in metabolism of LDL).

g) VLDL particles also transfer phospholipids (PL) and TG to HDL. At the same time HDL transfer cholesterol esters (CE) to VLDL. These reactions are catalyzed by cholesterol ester transfer protein.

Summary of VLDL metabolism:
1-Site of synthesis: Liver.
2-Functions: transport lipids mainly TG from liver to peripheral tissues.
3-Structure:
a-Main lipids: triacylglycerols. It contains also cholesterol, phospholipids.
b-**Proteins:** (10%), apo B_{100} and receives apo CII and apo E from HDL.
4-catabolism: TG are hydrolyzed by lipoprotein lipase (that is activated by apo CII). The remaining parts are IDL, which are then converted into LDL by transferring phospholipids, apo CII and apo E to HDL.



Metabolism of VLDL.

5. **Disorders of VLDL metabolism:**
   a) **Fatty liver:** This is an accumulation of abnormal amounts of fat in liver. It occurs when there is excess triacylglycerols synthesis in the liver, which is then excreted in the form of VLDL.

F. Metabolism of low density lipoproteins (LDL):

1. **Site of synthesis**: circulation, from VLDL.
2. **Function**: The primary function of LDL particles is to provide cholesterol to the peripheral tissues. **They do so through**:
   a) Deposition of free cholesterol on cell membranes.
   b) By binding to receptors on cell membranes that recognize apo B_{100}.
3. **Structure:**
   a) Lipids: cholesterol, cholesteryl esters, and phospholipids.
   b) Apoproteins (22%): include apo B_{100}.
4. **Catabolism:**
   a) The numbers between brackets refer to the corresponding numbers on the following figure:

   [1] The LDL receptors are negatively charged glycoprotein molecules, made by the DNA of the cell. They are grouped in pits in cell membranes. The intracellular side of the pits is coated with a protein called **clathrin.**

   [2] After binding with receptors, the LDL are internalized as intact particles by endocytosis.

   [3] The vesicle containing the LDL rapidly loses its clathrin coat and fuses with other similar vesicles, forming large vesicle called **endosomes.**

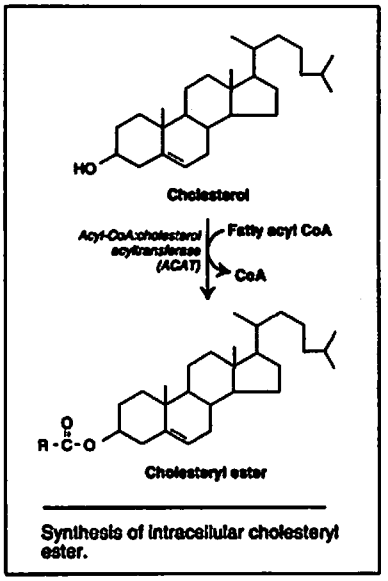
   [4] The pH of the contents of endosomes falls, allowing separation of the LDL from its receptors. The receptors then migrate to one side of the endosome while the LDL stays free within the lumen of the vesicle.

   [5] The receptors can be returned to the cell membrane. The lipoprotein remnants are hydrolyzed by lysosomal enzymes releasing cholesterol, amino acids, fatty acids and phospholipids.

   b) **If cell contains oversupply of cholesterol** : from LDL, HDL or chylomicron remnants, the cholesterol amount can be decreased and regulated by one of the following mechanisms:
      1) Oversupply of cholesterol inhibits HMG-CoA reductase activity so that cholesterol synthesis will be decreased.

2) Oversupply of cholesterol can stimulate a liver enzyme called: **acyl CoA cholesterol acyl transferase (ACAT):** ACAT transfers a fatty acid from fatty acyl CoA to cholesterol to form cholesteryl ester that can be stored inside the cell for future use.

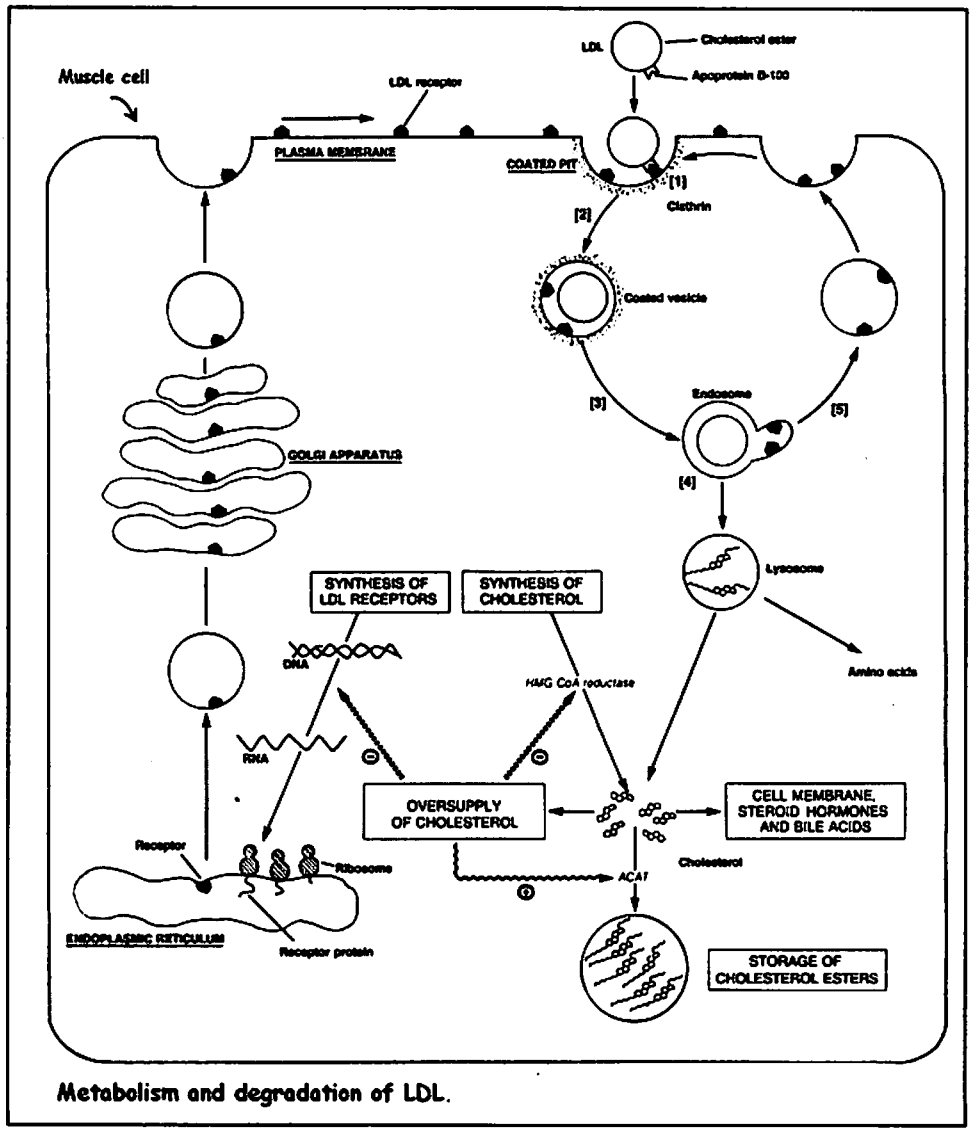
3) Oversupply of cholesterol inhibits the synthesis of new LDL receptor proteins, so that further entry of LDL cholesterol into the cell is limited.



Synthesis of intracellular cholesteryl ester.

5. Disorders of LDL metabolism:

a) **Type II familial hypercholesterolemia.:** LDL receptors are deficient in tissues and liver. Usually associted with atherosclerosis.

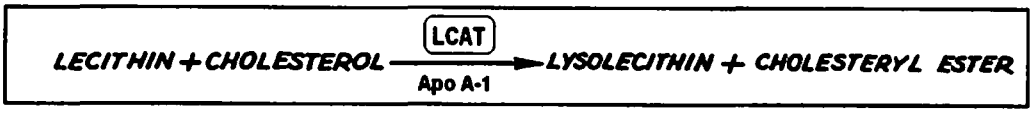


Metabolism and degradation of LDL.

SUMMARY OF LDL METABOLISM:
1-SITE OF SYNTHESIS: circulation from VLDL.
2-FUNCTION: LDL particles provide cholesterol to peripheral tissues.
3-STRUCTURE:
a- Lipid contents: cholesterol, cholesterol esters and phospholipids.
b- Protein contents: (22%), apo B_{100}.
4-CATABOLISM:
LDL apo B_{100} are recognized by tissue receptors. After binding with receptors, the LDL are internalized by endocytosis. Inside cells LDL are separated from receptors and hydrolyzed by lysosomal enzymes releasing cholesterol, amino acids, fatty acids and phospholipids.
***If the cell contains oversupply of cholesterol from LDL, HDL or chylomicron remnants, the cholesterol amount can be decreased by:**
a-Inhibition of HMG-CoA reductase ➔ Inhibition of cholesterol synthesis.
b-Stimulation of ACAT enzyme ➔ Cholesterol ester.
c-Inhibition of synthesis of LDL receptors ➔ inhibition of LDL uptake by cells.

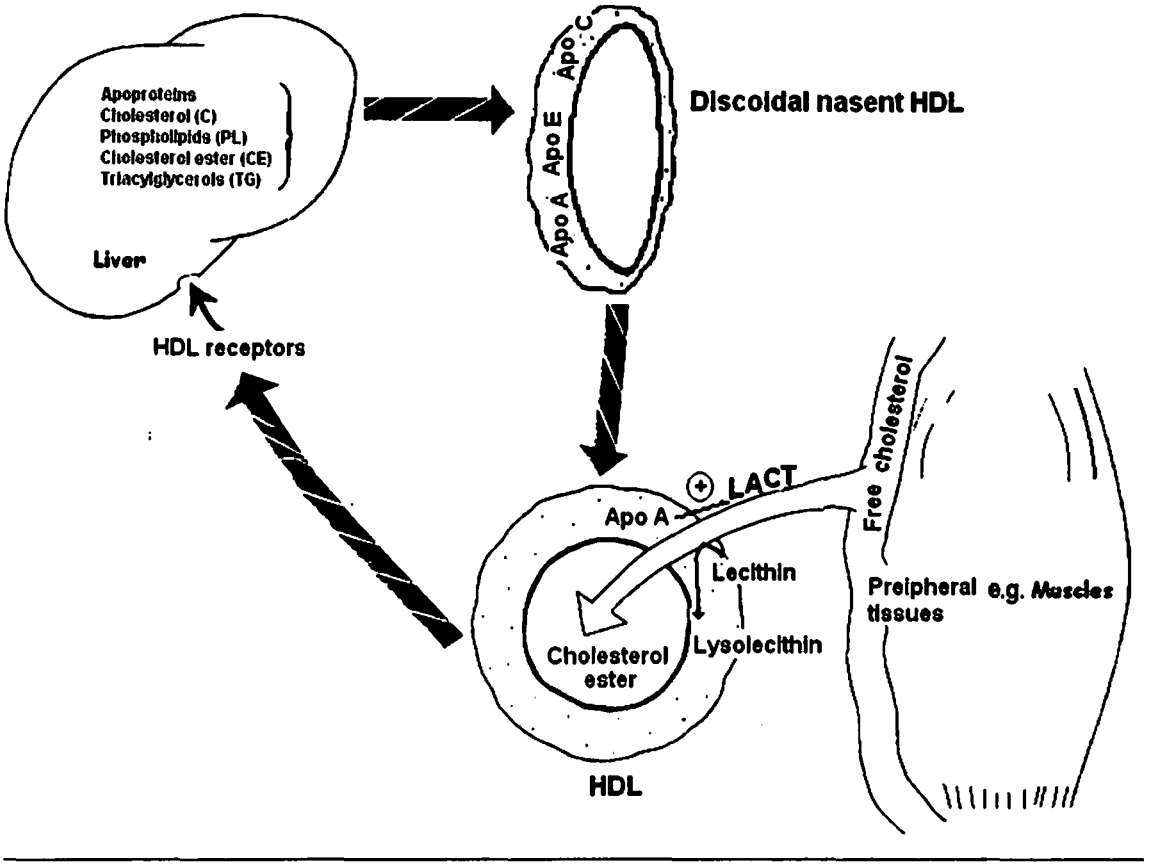
G. Metabolism of high density lipoproteins (HDL):

1. **Site of synthesis**: liver.
2. **Functions:**
   a) Act as reservoir of apo C-II that is transferred to chylomicrons and VLDL to activate lipoprotein lipase enzyme.
   b) Remove free (unesterified) cholesterol from extrahepatic tissue and esterifying it, using a plasma enzyme called: **lecithin, cholesterol acyl transferase (LCAT)**. The apo A-1 OF HDL activates LCAT.



   c) HDL particles carry cholesterol esters to:
      1) VLDL and LDL.
      2) Liver where the HDL is hydrolyzed and cholesterol released.
3. **Structure:**
   a) Lipids: Mainly phospholipids together with esterified & unesterified cholesterol.
   b) Proteins (50%): Include Apo A-1, Apo C and Apo E.
4. **Catabolism :**
   a) A newly secreted HDL are disc shaped particles containing mainly unesterified cholesterol and phospholipids.
   b) HDL is converted into spherical particles by accepting unesterified cholesterol from peripheral tissues (surface of cell membranes).
   c) Once the free cholesterol is taken up, it is immediately esterified by LCAT. The resulting cholesterol ester is very hydrophobic, so it remains in HDL and cannot be transferred to peripheral tissues.
   d) The liver takes up HDL particles, where the cholesteryl esters are hydrolyzed. The released cholesterol may undergo:

1) Binding with apoproteins to form lipoproteins.
2) Converting to bile acids.
3) Secreted into the bile to be removed from the body.



Metabolism of HDL.

Professor Said Oraby

Summary of HDL metabolism:

1-Site of HDL synthesis: Liver.

2-Functions:

a-Contain Apo C-II, which activates lipoprotein lipase that hydrolyzes TG.

b-Remove cholesterol from peripheral tissues and esterified it by LCAT enzyme ➔**Cholesterol esters.**

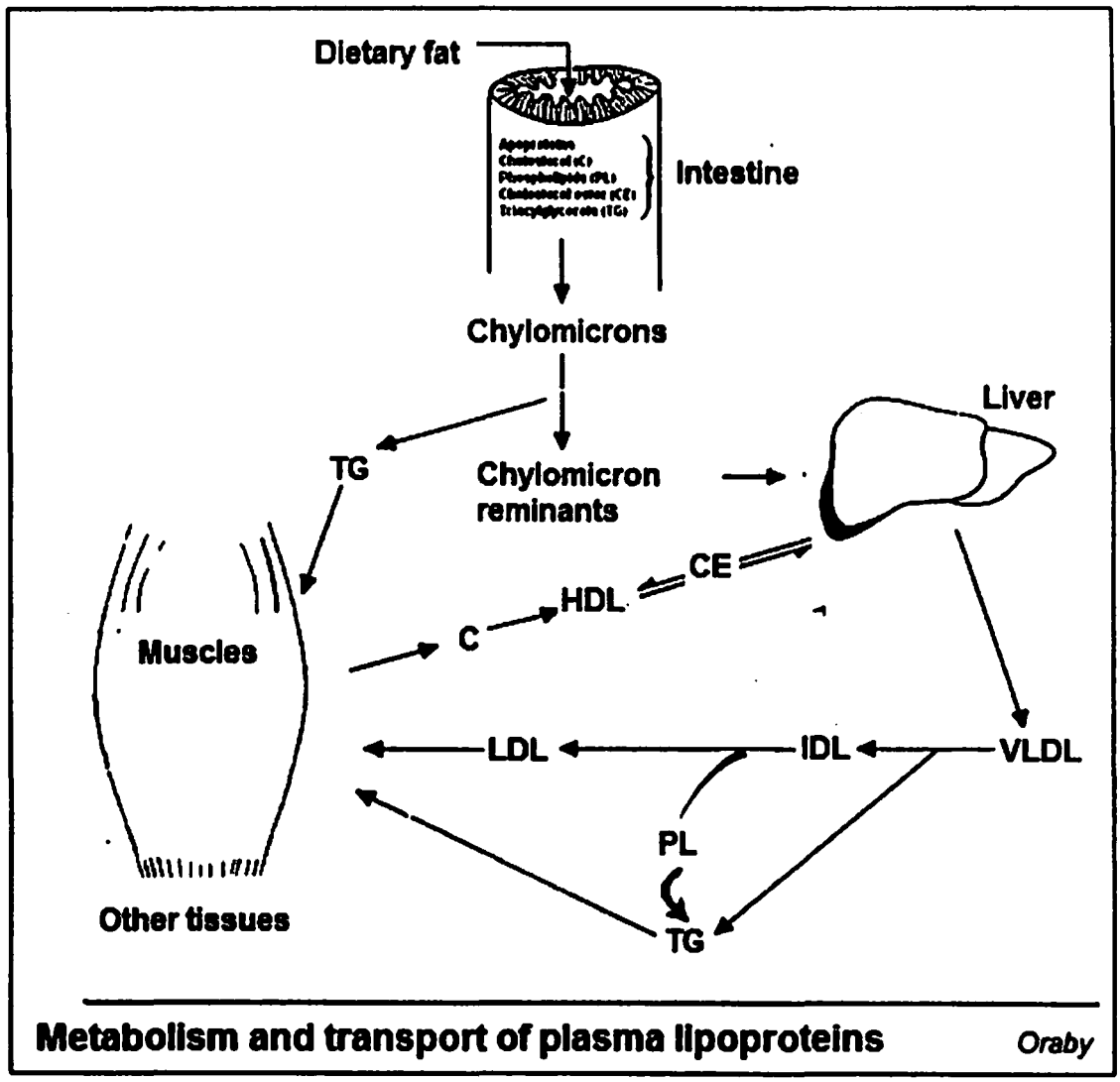
c- Carry cholesterol esters to VLDL & chylomicrons to the liver.

3-Structure:

a-Main lipids: cholesterol (free and esterified) and phospholipids, mainly lecithin.

b-**Proteins**: (50%), apo A-1(which activates LCAT), apo CII (which activates lipoprotein lipase and E (which is recognized by hepatic receptors)

4-Catabolism: HDL accepts unesterified cholesterol from peripheral tissues. Then HDL particles (by LCAT enzyme) esterify cholesterol into cholesterol esters. Then HDL is taken up by the liver cells where Cholesterol esters are released inside them to be utilized in the formation of lipoproteins or excreted in bile.



Metabolism and transport of plasma lipoproteins *Oraby*

H. Catabolism of free fatty acids (FFA):

1. Free fatty acids (FFA) are transported conjugated with albumin.
2. Plasma FFA are produced by lipolysis in adipose tissue and by the action of lipoprotein lipase in chylomicrons and VLDL.
3. Their level increases in all conditions associated with excessive lipolysis e.g. fasting, diabetes mellitus...etc.
4. Different tissues oxidize them to give energy.
5. In cases of excessive lipolysis e.g. uncontrolled diabetes mellitus, FFA are partially oxidized and produce ketone bodies.

I. *Lipoprotein (a):*

1. Lipoprotein A, commonly called Lp(a), is a major independent risk factor for cardiovascular disease. The optimum laboratory level should be under 20 mg/dl

2. Lipoprotein (a) is derived in the blood from low density lipoprotein (LDL) molecules and glycoprotein molecules called apolipoprotein-a (apo-a). Plasma apo-a is secreted by the liver.

3. High Lp (a) in blood is present in familial hypercholesterolemia, and is a risk factor for coronary heart disease (CHD), cerebrovascular disease (CVD), atherosclerosis, thrombosis, and stroke.

III. *APOPROTEINS (APOLIPOPROTEINS):*

A. **Definition:** These are proteins (globulins) present in association with plasma lipids to form lipoproteins.

B. **General role (functions):**

1. Apolipoproteins form with lipids water-soluble compounds, so they help transport of lipids between tissues.
2. Some apoproteins activate certain enzymes e.g. Apo C II activates lipoprotein lipase and Apo-A-1 activates LCAT.
3. They act as ligands (connection) for interaction of lipoprotein with their receptors in tissues i.e. receptors of lipoproteins in tissues can recognize lipoproteins through their apoproteins e.g. apo B_{100} for LDL receptors

C. **Classification (Types):**

Apolipoproteins	Lipoproteins	Functions
Apo A-1	HDL	• Activator of LCAT • Ligand for HDL receptor
Apo B_{-48}	Chylomicrons-Chylomicron remnants	• Synthesized by intestine • Ligand for chylomicron remnant receptors in liver
Apo B_{-100}	LDL – VLDL - IDL	• Synthesized by liver • Ligand for LDL receptors
Apo CII	Chylomicrons - VLDL - HDL	• Activator of lipoprotein lipase
Apo D	HDL	• May act as lipid transfer protein.
Apo E	Chylomicrons-Chylomicron remnants – VLDL - HDL	• Ligand for chylomicron remnant receptors in liver

***Note:** Apo B_{100} is synthesized in liver and is one of longest polypeptide chain (4530 amino acids), while Apo B_{48} is synthesized in intestine and is 48% as large as Apo B 100.*

IV. *DYSLIPOPROTEINEMIAS*: (DISORDERS OF PLASMA LIPOPROTEINS):

A. **Hyperlipoproteinemias:**

1. **Primary hyperlipoproteinemia:**

a) **Type I or familial lipoprotein lipase deficiency:** Chylomicrons and VLDL are markedly increased

b) **Type II or familial hypercholesterolemia:**

1) LDL receptors are deficient in tissues and liver
2) Usually associated with atherosclerosis.

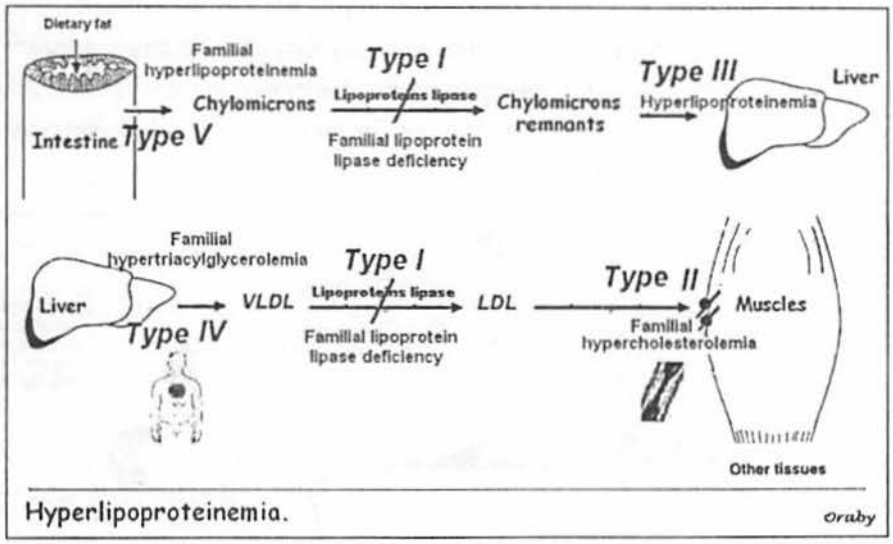
c) **Type III or hyperlipoproteinemia:**

1) Clearance of chylomicron remnants and VLDL remnants is deficient.
2) Chylomicrons and VLDL remnants are increased.

d) **Type IV or familial hypertriacylglycerolemia:**

1) Due to overproduction of VLDL.
2) Usually associated with coronary heart disease, type II diabetes and obesity.

e) **Type V or familial hyperlipoproteinemia:** Due to overproduction of VLDL and chylomicrons.



Hyperlipoproteinemia. Oraby

2. <u>Secondary hyperlipoproteinemia</u>: These abnormalities associated with other diseases as:

a) Diabetes mellitus
b) Hypothyroidism.
c) Nephrotic syndrome.
d) Obesity.
e) Obstructive jaundice.

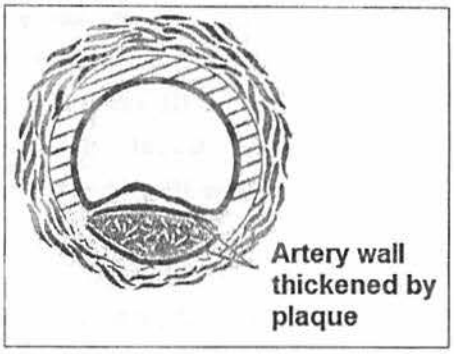
B. <u>Hypolipoproteinemias</u>:

1. <u>Abetalipoproteinemia</u>: Characterized by absence of LDL (β-lipoprotein). It is associated with low concentrations of chylomicrons and VLDL.
2. <u>Tangier disease:</u>

a) It is due to deficiency of **LCAT** enzyme.
b) It is characterized by low concentration of HDL with accumulation of cholesterol in tissues.

V. *Plasma lipoproteins and atherosclerosis:*

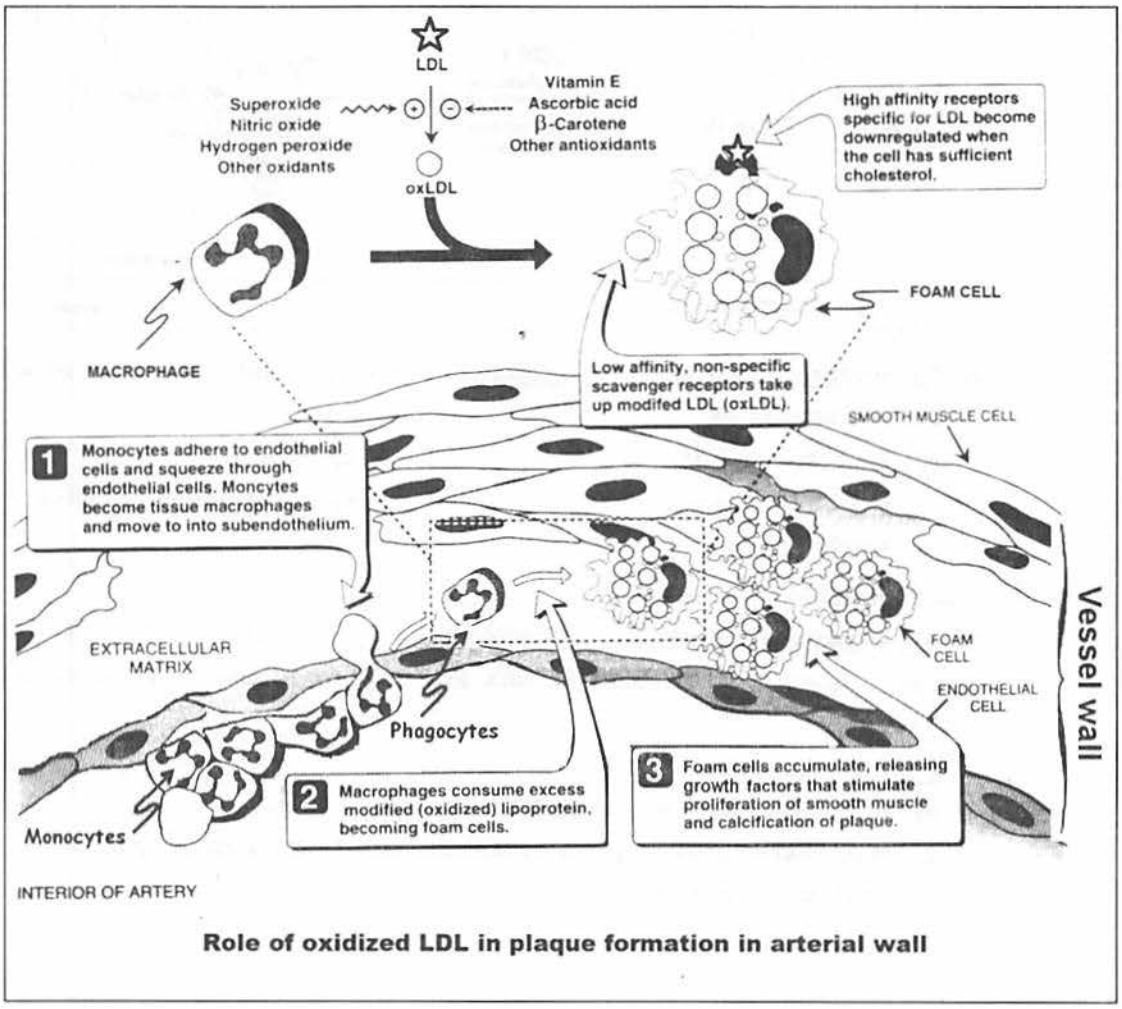
A. Definition: Atherosclerosis is a chronic disease in which deposits of cholesterol, cholesteryl esters and cellular debris accumulate in the inner surfaces of large and medium sized arteries forming plaque. As the disease progresses, the deposits reduce or even, stop the flow of blood causing **coronary artery disease** or **cerebral artery disease**.



NOTE: If atherosclerosis affects and blocks coronary artery branches of the heart, it may lead to death of heart cells and this is called myocardial infarction.

B. Causes of atherosclerosis:

1. Diseases associated with prolonged elevated levels of VLDL, IDL and LDL e.g. diabetes mellitus; hypothyroidism and hyperlipidemia.
2. Oxidation of LDL by superoxide, hydrogen peroxide and other oxidants into oxidized LDL ➔ Plaque formation and atherosclerosis. See the following diagram:



Role of oxidized LDL in plaque formation in arterial wall

C. **Risk factors for atherosclerosis:** Since the LDL represents the transport of cholesterol to the tissues and HDL represents the removal of cholesterol from tissues thus:

1. **LDL/HDL ratio** helps in predicting atherosclerosis and coronary heart disease:

   ↑ LDL/HDL → Atherosclerosis

   ↓ LDL/HDL → No atherosclerosis

2. ***Estrogens and exercises cause increase HDL***, thus premenopausal women and persons doing exercises (jogging) appear to be protected from coronary heart diseases. Also, diet rich in polyunsaturated fatty acids lowers blood cholesterol.

Summary of lipase enzymes:

Enzyme	Origin	Site of action	Function	Special properties
Gastric lipase	Stomach	Stomach	Degrades dietary triacylglycerols in infants	Needs acid pH
Pancreatic lipase	Pancreas	Small intestine	Degrades dietary triacylglycerols (removes fatty acid from carbon 1 and 3, leaving 2-monoglycerol)	Needs colipase
Lipoprotein lipase	Blood and extrahepatic tissues	Surface endothelial cells lining the capillaries	Degradeds triacylglycerol circulating in chylomicrons or VLDL, releasing FA and glycerol	Can be released into plasma by heparin; activated by apoprotein C-II
Hormone sensitive lipase	Adipose tissue	Adipose tissue	Degradation of stored triacylglycerols	Activated by cAMP
Acid lipase	Most tissues	Lysosomes	Removes fatty acids from lipids taken into cells during phagocytosis	Needs acid pH
Hepatic lipase	Liver	Liver	Removes fatty acids from chylomicron remnants and HDLtaken by hepatic cells.	

VI. *Role of liver in fat metabolism:*

1. Fatty acid synthesis and oxidation.
2. Synthesis and esterification of cholesterol.
3. Formation of lipoproteins.
4. Formation of phospholipids.
5. Formation of ketone bodies.
6. Formation of bile salts.
7. Storage of fat soluble vitamins.
8. Detoxication of steroid hormones.

Fatty liver

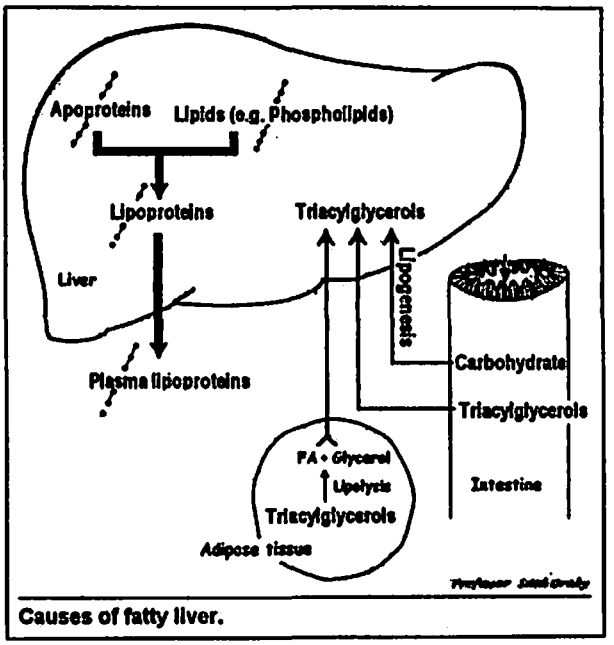
I. ***Definition of fatty liver:*** This is an accumulation of abnormal amount of fat in the liver for a long time with subsequent compression of liver cells. This results in liver fibrosis and impairment of liver function.

II. ***Causes:***

- Overmobilization of fat from extrahepatic tissues to the liver.
- High carbohydrate diet.
- Undermobilization of fat from the liver to the plasma.

A. **Causes of overmobilization of fat from extrahepatic tissues to the liver:**

1. After high fat diet.
2. Excessive lipolysis due to low carbohydrate diet, starvation and diabetes mellitus.



Causes of fatty liver.

B. **High carbohydrate diet**: On high carbohydrate diet, liver is first saturated with glycogen, then any further amount of carbohydrate will be converted to triacylglycerols (lipogenesis).

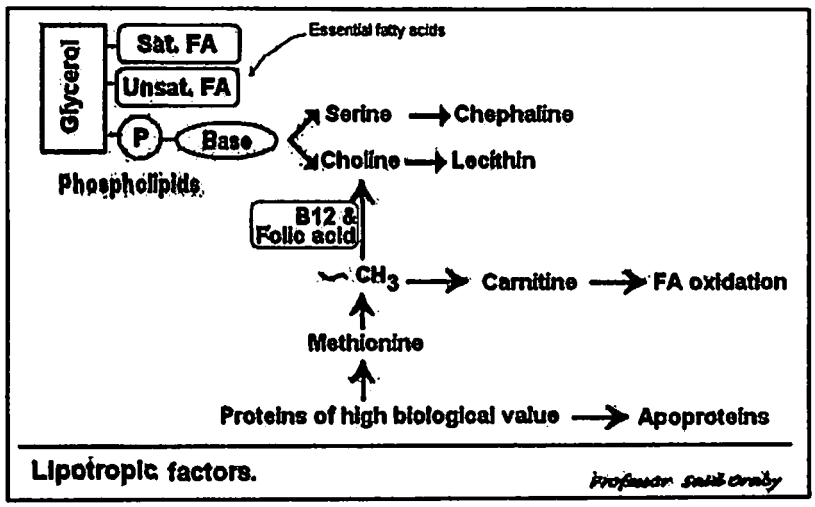
C. **Causes of under-mobilization of fat from liver to the plasma**: This is due to deficiency of any factor essential for plasma lipoproteins formation. These factors are:

1. **Deficiency of lipotropic factors:** see below
2. Decreased synthesis of apoprotein (apolipoprotein).
3. Failure in formation of phospholipids.
4. Failure in conjugation of apoproteins with triacylglycerols or phospholipids
5. Failure in secretion of lipoproteins from liver to plasma.
6. **Liver poisons:** As carbon tetrachloride, chloroform, lead and arsenic. They cause fatty liver by:
   a) Inhibition of formation of apoprotein.
   b) Inhibition of conjugation of apoprotein with lipids.
   c) Inhibition of secretion of lipoprotein.

7. **Alcoholism:** Ethanol stimulates lipogenesis, inhibiting fatty acid oxidation. This leads to accumulation of fat in liver with subsequent fatty liver.

D. Lipotropic factors:

1. **Definition:** Lipotropic factors are substances that help the mobilization of fat from the liver.
2. **They include:**
   a) **Substances enter in structure or help the formation of phospholipids.**
      1) Essential fatty acids (polyunsaturated fatty acids).
      2) Choline, enters in structure of lecithin, sphingomyelin.
      3) Inositol, enters in structure of lipositol.
      4) **Amino acids:** as
         i- Methionine which is a methyl donor essential for choline formation.
         ii- Serine which enters in the structure of cephalin.
      5) **Vitamins:** Vitamin B_{12} and folic acid have a role in synthesis and transfer of methyl group (CH_3).



Lipotropic factors.

b) **Proteins of high biological value:**
   1) These proteins include all essential amino acids which enter in structure of apoproteins in the liver.
   2) These proteins include methionine. Methionine is essential for:
      i- Choline formation.
      ii- Carnitine: a substance essential for fatty acid oxidation.

Summary of lipids pathways

	Lipogenesis	Lipolysis	FA oxidation	FA synthesis	Ketone bodies oxidation	Ketone bodies synthesis	Cholesterol
Definition	Acyl CoA + Glycerol-3-p to TG	TG to FA + glycerol	FA to Acetyl CoA to Krebs' cycle to give energy	Acetyl CoA to Palmitate	Acetacetate, β-hydroxybutyric acid and acetone		Acetyl CoA to cholesterol
Location	Cytosol Liver &adipose t.	Cytosol Of adipose tissue	Mitochondria of liver ..etc	Cytosol of liver ..etc	Mitochondria of liver	Mitochondria of extrahepatic tissue	Cytosol Liver ..
Steps	See	See	See	See	See	See	See
Functions	Synthesis of TG	----	Energy Acetyl CoA Ketone bodies	Synthesis of FA	Energy		Cell membrane Vitatmin D Bile acids Steroid Hs.
Key enzymes	Acyl CoA synthetase Glycerol-3-p DH Glycerokinase	HSTG lipase	----	Acetyl CoA carboxylase	HMG CoA synthase	Thiophrase	HMG CoA reductase
Regulation	Hormonal	Phosphorylation dephosphoryla-tion	----	Phosphorylation dephosphoryla-tion Long chain acyl CoA Hormonal	Hormonal regulation		Phosphorylation dephosphorylation Feed back inhibition Feed back regulation drugs
Diseases	-----	-----	Refsum's disease		Ketosis		Hyper and hypocholesterolemia

TG = Triacylglycerols, FA = fatty acids, DH = dehydrogenase, HSTG lipase = Hormone sensitive triacylglycerol lipase.

Chapter 4 *Integration of Metabolism*

I. *INTERCONVERSION OF MAJOR FOODSTUFFS:*

A. Glucose can be converted into fatty acids:

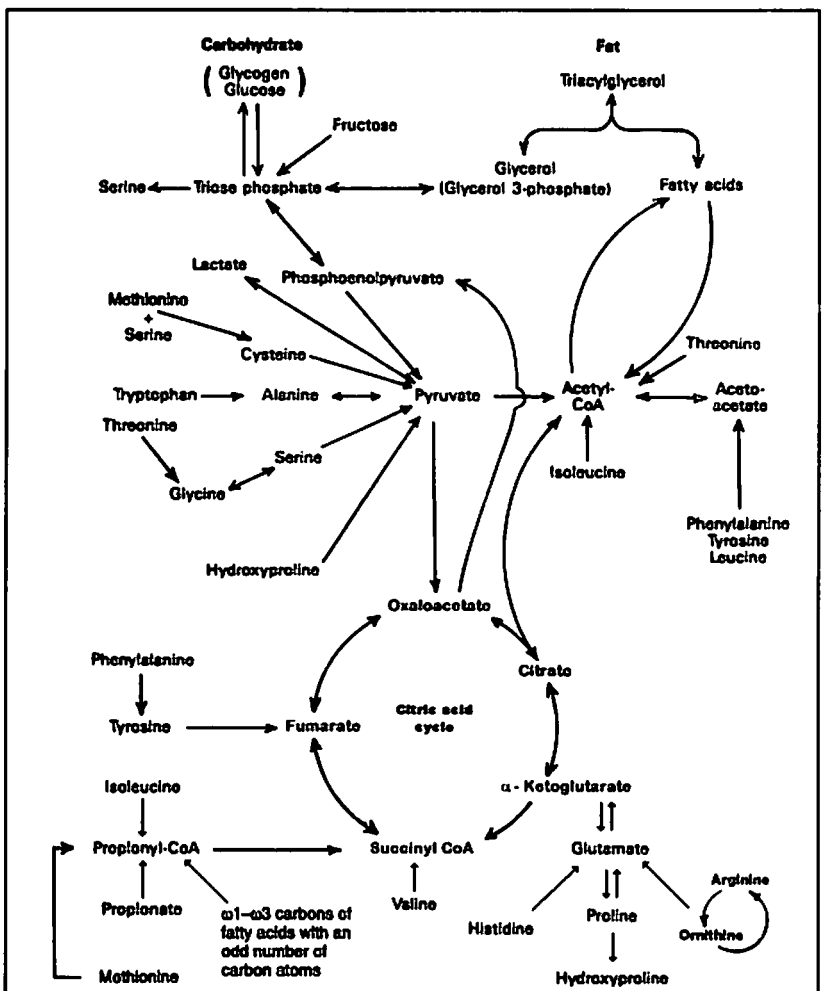
Glucose —Pyruvate dehydrogenase→ Acetyl CoA → Fatty acid

B. Fatty acids **never** converted into glucose? Because pyruvate dehydrogenase reaction is irreversible.

C. Only oxidation of Odd number fatty acids ➔ Propionyl CoA ➔ Succinyl CoA ➔ Glucose (by gluconeogenesis).

D. Glycerol ➔ (derived from triacylglycerols) ➔ Glycerol-3-phosphate ➔ Dihydroxyacetone phosphate ➔ Glucose.

E. The carbon skeletons of ketogenic amino acids ➔ Acetoacetate ➔ Acetyl CoA (in extrahepatic tissue).



Interconversion of the major foodstuffs

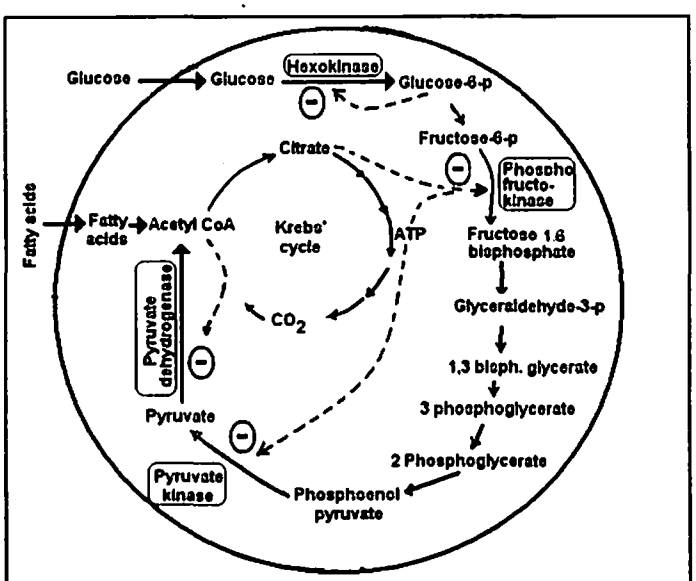
II. *FUELS USED BY TISSUES:*

A. Types:

1. Glucose.
2. Fatty acids.
3. Ketone bodies.
4. Glycerol.
5. Lactate.
6. Carbon skeleton of some amino acids.

B. Order of preference for oxidation of fuels:

1. Under conditions of carbohydrate deficiency, available fuels are oxidized in the following order of preference:
   a) Ketone bodies.
   b) Free fatty acids.
   c) Glucose.
2. The preferential utilization of ketone bodies and free fatty acids **spares glucose** for its essential functions. **This sparing can be done by:**
   a) Insulin release is decreased ➔ ⬇ Glucose uptake by muscles and inhibition of key enzymes of glucose oxidation.
   b) Fatty acids and ketone bodies oxidation ➔ Acetyl CoA ➔ Inhibition of pyruvate dehydrogenase complex and other enzymes of glycolysis (see the diagram).
   c) Fatty acids ➔ Acetyl CoA ➔ Citrate ➔ Inhibition of phospho-fructokinase-1 by citrate. CAC also produce ATP ➔ Inhibition of phosphofructokinase and Pyruvate kinase enzymes.
   d) Allosteric inhibition of hexokinase by glucose-6-phosphate.



The mechanism of inhibition of glucose oxidation by fatty acid oxidation.

Professor Said Oraby

C. **Importance of glucose as a source of energy:**

Even at times of glucose shortage, minimal supply of glucose is required for:

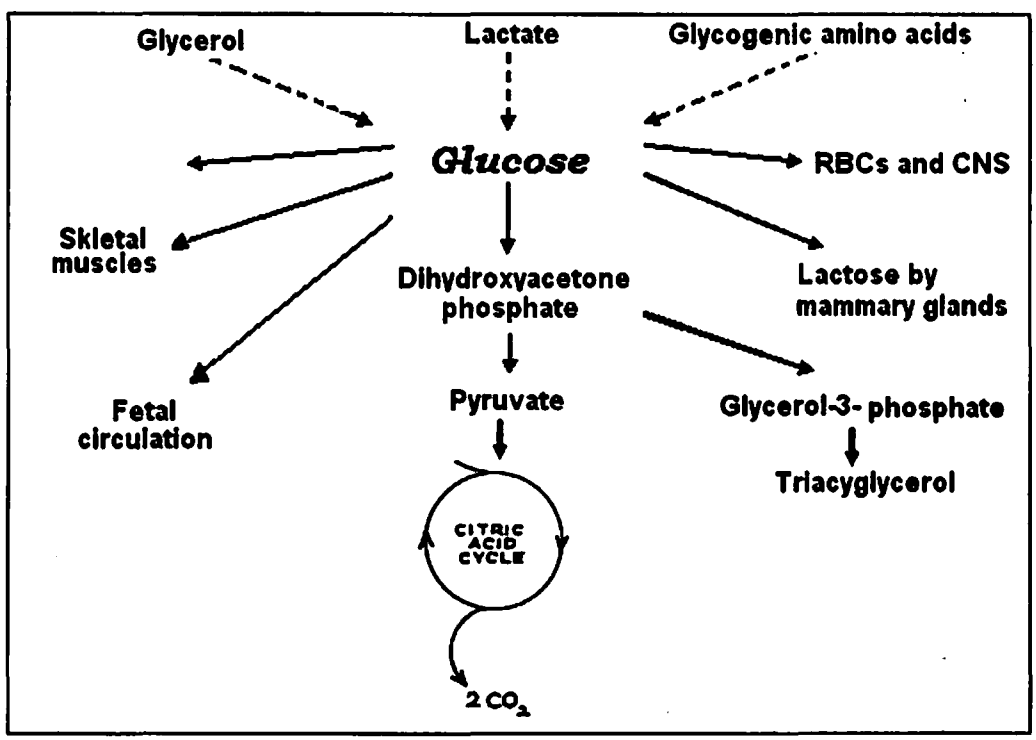
1. **RBCs and CNS:** There are some tissues, which depend **only** on glucose as the source of energy e.g. CNS And RBCs.
2. **Skeletal muscles:** Glucose is the only fuel for skeletal muscles during **exercises** (under anaerobic conditions).
3. **Lipogenesis:** Glucose is the main source of glycerol-3-phosphate in tissues deficient of glycerol kinase (as adipose tissue) → Lipogenesis.

Glucose → Dihydroxyacetone phosphate → Glycerol-3-phophosphate → Lipogenesis.

4. **Citric acid cycle:** A minimal supply of glucose is necessary in extra hepatic tissues to maintain oxaloacetate concentration → Essential for integrity of citric acid cycle.
5. **Lactose:** Glucose is the source of milk sugar (lactose) in mammary gland.

D. **Sources of glucose:**

1. At times of glucose shortage e.g. starvation, glucose is supplied through gluconeogenesis. The **gluconeogenic substances** include:
   a) **Lactate:** formed in RBCs and skeletal muscles.
   b) **Glycerol:** formed in adipose tissue.
   c) **Glucogenic amino acids.**



E. **Fuels used by active tissues:**

1. **Heart:** Uses all fuels.
2. **Muscles:**
   a) **During rest:** Utilize fatty acids.
   b) **During exercises:** Utilize glucose.
   c) **During starvation:** utilize fatty acids, ketone bodies and amino acids (after transamination inot α-keto acids).
3. **Liver:**
   a) **In fed state:** utilizes glucose, fatty acids and amino acids.
   b) **During starvation:** utilizes fatty acids and amino acids.
   c) Liver never **utilizes ketone bodies** because it does not contain enzymes of ketolysis.
4. **Brain:**
   a) **General:** Glucose.
   b) **Starvation:** Ketone bodies.
5. **RBCs:**
   a) **Glucose:** anaerobic oxidation (glycolysis).
   b) RBCs never oxidize ketone bodies because RBCs **contain no mitochondria.**
6. **Kidneys:**
   a) **General:** utilize glucose and fatty acids.
   b) **Starvation:** utilize amino acids and fatty acids.

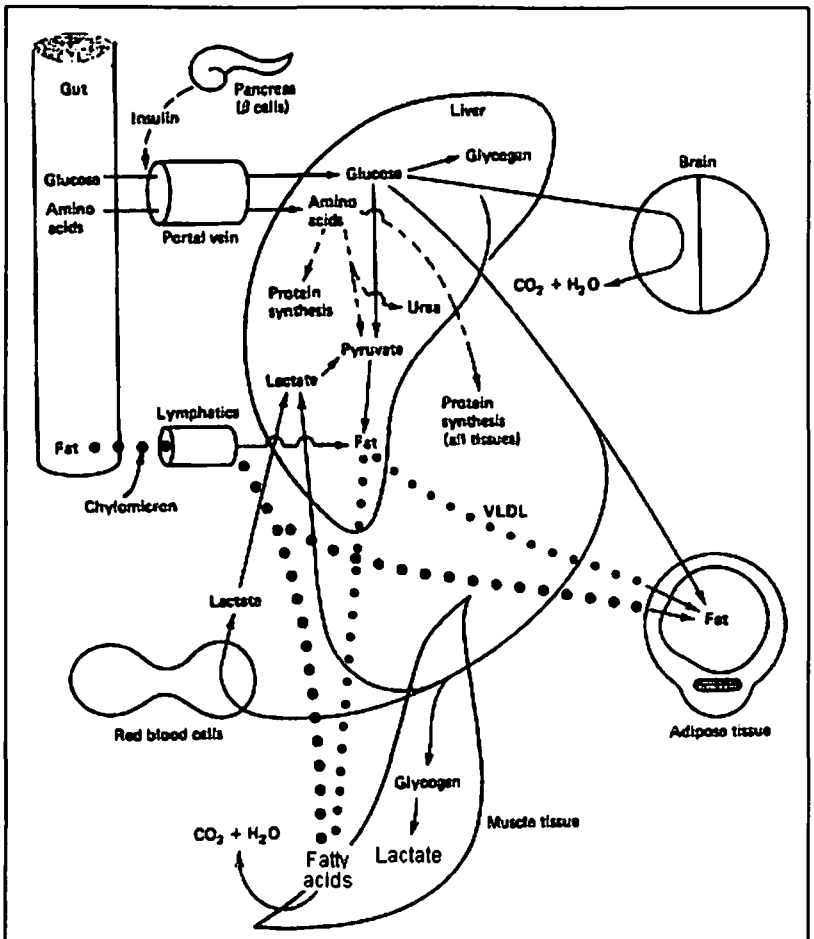
Fed - Starvation cycle

I. *THE FED (OR ABSORPTIVE) STATE:*

After meal, blood glucose, amino acids and chylomicrons levels increase ➔ ↑ Insulin and ↓ Glucagon.

A. **The fate of glucose in fed (absorptive) state:**

1. **The fate of glucose in the liver:** Liver cells either oxidize glucose or convert it into glycogen and triacylglycerols:
   a) **Glucose** is oxidized into CO_2 and H_2O → Energy needs of the liver.
   b) **Excess glucose** is stored in the liver as glycogen, which is used during periods of early fasting (between meals) to maintain normal blood glucose.



Disposition of glucose, amino acids, and fat by various tissues in the well-fed state.

c) **More excess glucose** (after glycogen formation) can be converted into fatty acids and glycerol → triacylglycerols → released from the liver into the blood as VLDL.

2. **The fate of glucose in other tissues:**

a) **Brain,** which depends on glucose for its energy, **oxidizes glucose** → CO_2 and H_2O → Energy.

b) **Red blood cells,** lacking mitochondria, **oxidize glucose anaerobically** → Pyruvate → **Lactate** → Released into blood.

c) **Muscle cells:** Glucose transport across cell membrane is stimulated by insulin. It undergoes:

(i) **Glucose oxidation** → CO_2 and H_2O → Energy for contraction.

(ii) **Glycogen synthesis** → Stored and used during contraction.

d) **Adipose cells:** Glucose transport across cell membrane is stimulated by insulin. It undergoes:

(i) **Glucose oxidation** ➔ CO_2 and H_2O ➔ Energy for contraction.

(ii) **Triacylglycerols synthesis:** Glucose is converted to glycerol ➔ Triacylglycerols.

B. **The fate of lipoproteins in the fed state:**

1. The triacylglycerols of **chylomicrons** (produced from dietary fat) and **VLDL** (produced from glucose by the liver) are hydrolyzed in capillaries by **lipoprotein lipase** → Glycerol and fatty acids.
2. **fatty acids and glycerol** are taken up by adipose tissue → Triacylglycerols ➔ Stored.

C. **The fate of amino acids in the fed state:** Amino acids from dietary proteins enter cells and are used for:

1. **Protein synthesis:**
2. Synthesis of **nitrogenous compounds** such as heme, creatine, phosphate, epinephrine and the bases of DNA and RNA.
3. Oxidation to generate **ATP (Amino acid** ➔ α-Ketoacids ➔ Oxidation ➔ Energy).

II. *THE FASTING (STARVATION) STATE:*

A. **Introduction:**

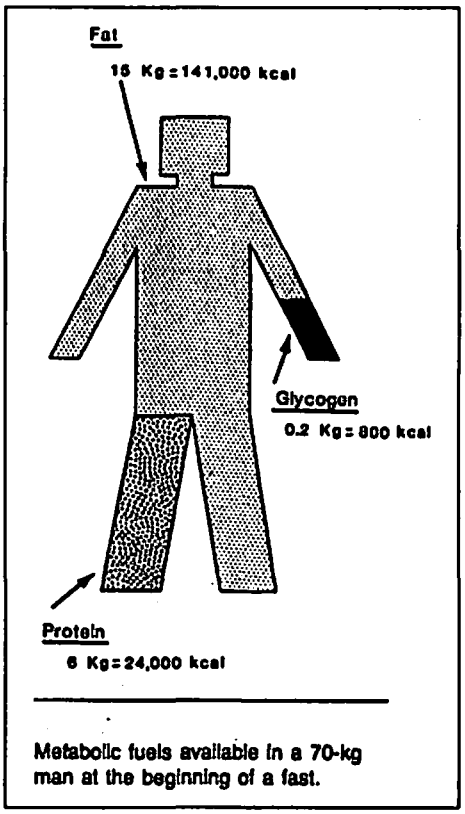
1. Starvation may result from:
   a) Inability to obtain food.
   b) The desire to lose weight rapidly.
   c) In clinical situations in which an individual cannot eat because of trauma, burns...etc.
   d) As a mean of political strike.
2. The recorded time for a man survived while starving was 54 days.

B. **Fuel store in human:** At the beginning of starvation, the metabolic fuels available in a normal 70 Kg man are shown in the following table and figure:

Stored fuel	Tissue	Amount	Kcal
Glycogen	Liver and muscles	470 grams	1880
Fats	Adipose tissue	15 kg	141000
Proteins	Muscles	6 kg	24000
Glucose	Body fluids e.g. blood	20 grams	80

Note: energy production from different food stuffs is as follows:

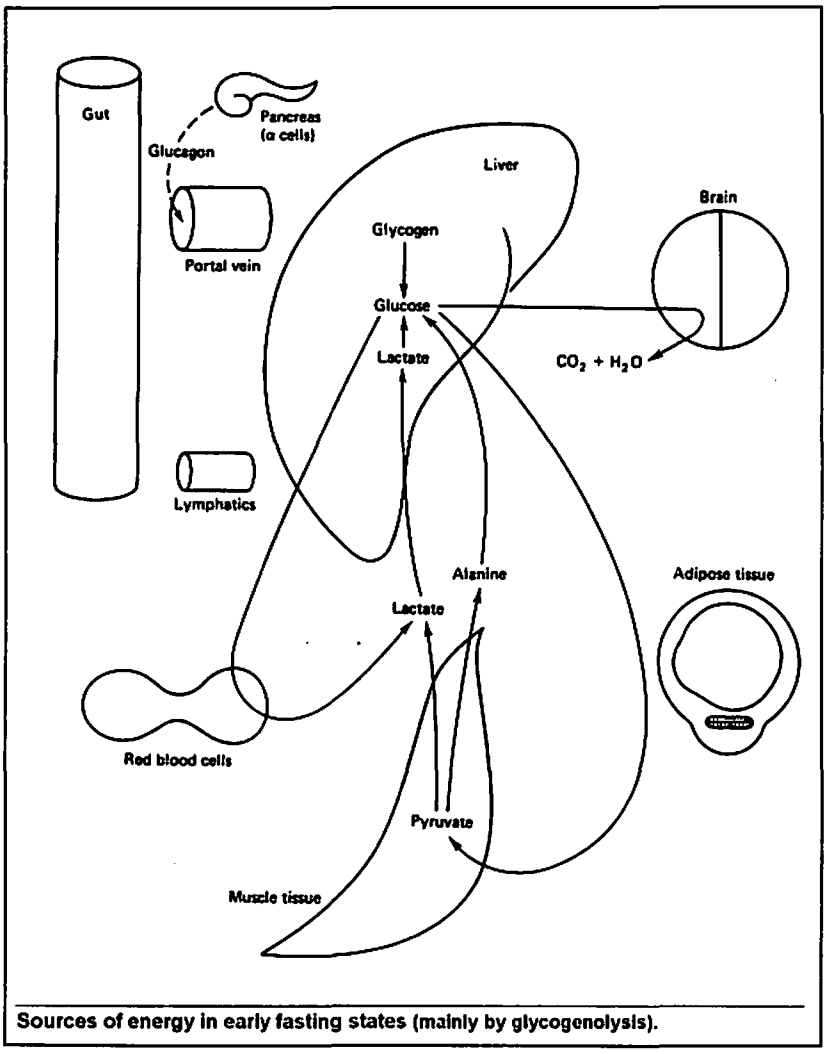
- Carbohydrates: 4 Kcal/g.
- Proteins: 4 Kcal/g.
- Fat: 9 Kcal/g.



Metabolic fuels available in a 70-kg man at the beginning of a fast.

C. Mechanism of energy supplies:

1. As blood glucose levels decrease → ↓ insulin and ↑ glucagon → stimulating the release of fuels into the blood.
2. The **liver supplies glucose** and ketone bodies to the blood. The liver maintains blood glucose levels by glycogenolysis and gluconeogenesis and synthesizes ketone bodies from fatty acids supplied by adipose tissue.
3. **Adipose tissue:** triacylglycerols → releases fatty acids and glycerol. The fatty acids will undergo:
   a) **In tissues** → Oxidized to CO_2 and H_2O → Energy.
   b) **In liver:**
      (i) Fatty acid → Acetyl CoA → ketone bodies.
      (ii) Glycerol → gluconeogenesis.
4. Muscles release amino acids. The carbon skeletons are used by the liver for gluconeogenesis, and the nitrogen is converted to urea.



Sources of energy in early fasting states (mainly by glycogenolysis).

D. Liver in starvation:

1. **Carbohydrate metabolism:** The liver maintains blood glucose levels first by glycogen breakdown (glycogenolysis), then gluconeogenesis:
   a) **Increased glycogen breakdown (glycogenolysis):** ↑ glucagon and ↓ insulin → rapid mobilization of liver glycogen → Blood glucose. **Note** that liver glycogen is formed 3-4 hours after meal and is nearly **exhausted after 18 to 24 hours** of fasting,
   b) **Increased gluconeogenesis:**
      (i) The carbon skeletons for gluconeogenesis are derived primarily from **amino acids, glycerol and lactate.**
      (ii) Gluconeogenesis **begins 4 to 6 hours** after the last meal and becomes fully active, after **18 to 24 hours** (after exhaustion of liver glycogen).
      (iii) Gluconeogenesis plays an essential role in maintaining blood glucose during both overnight and prolonged fasting.

2. **Fat metabolism :**

a) **Increased fatty acid oxidation:** The oxidation of fatty acids derived from adipose tissue is the major source of energy in hepatic tissue in the post absorptive state.

b) **Increased synthesis of ketone bodies:**

(i) Liver is the site of synthesis and release of ketone bodies for use as fuels by peripheral tissues. Ketone bodies synthesis is increased when the concentration of acetyl CoA, produced from fatty acid metabolism exceeds the oxidation capacity of Krebs' cycle.

(ii) The availability of circulating ketone bodies is important in starvation because they can be used as fuel by most tissues including brain.

E. **Adipose tissue in starvation:**

1. **Carbohydrate metabolism:**

a) **Inhibition of glucose transport:** into the adipocyte and its subsequent metabolism. This is due to low levels of circulating insulin. This leads to decrease in fatty acid and triacylglycerol synthesis.

2. **Fat metabolism:**

a) **Increased degradation of triacylglycerols:**

(i) ↑Glucagon and ↓insulin and the release of epinephrine and norepinephrine → Activation of hormone sensitive lipase → ↑ Increased degradation of triacylglycerols.

(ii) **Increased release of fatty acids:**

- Fatty acids obtained from hydrolysis of stored triacylglycerol are released into the blood → They bound to albumin and transported to various tissues for use as fuel.
- The glycerol produces after triacylglycerol degradation → Liver → gluconeogenesis → Glucose.

F. **Skeletal muscles in starvation:**

1. **Carbohydrate metabolism:**

↓ Insulin → ↓ Glucose transport into muscle cells → ↓ Glucose oxidation.

2. **Fat metabolism:**

a) During the first two weeks of starvation, the muscle uses fatty acids from adipose tissue and ketones from the liver as fuel.

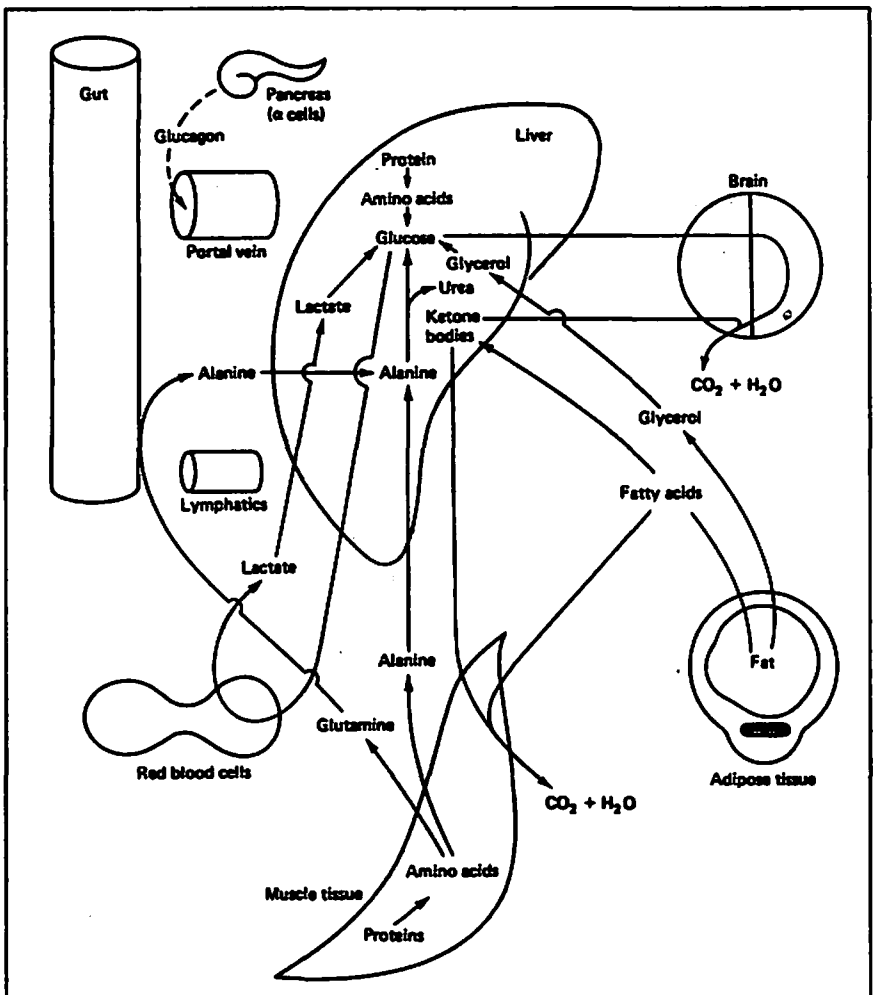
b) After about 3 weeks of starvation, muscle decreases its use of ketones and oxidizes fatty acids almost exclusively. This leads to a further increase in the already elevated level of circulating ketone bodies.

3. **Protein metabolism:**

a) During the first few days of starvation, there is a rapid breakdown of muscle protein providing amino acids that are used by the liver for gluconeogenesis. After several weeks of starvation, the rate of muscle breakdown decreases due to a decline in the need for glucose as a fuel for the brain.

G. Brain and starvation:

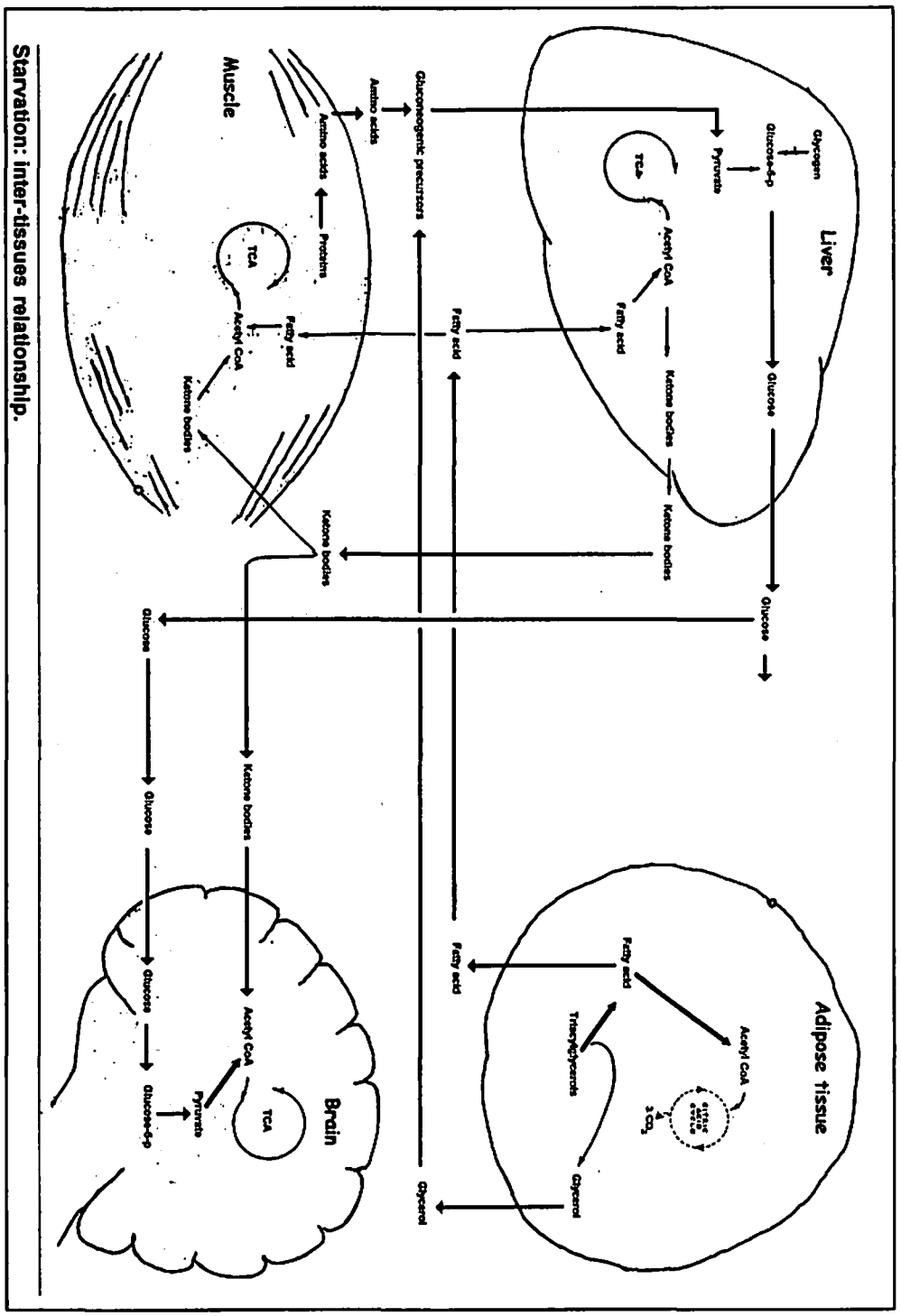
1. During the first days of starvation, the brain continues to use glucose exclusively . Blood sugar is maintained by hepatic gluconeogenesis from amino acids provided by the rapid breakdown of muscle protein.
2. In prolonged starvation (greater than 2-3 weeks), plasma ketones reach markedly elevated levels and are used as a fuel by the brain. This reduces the need for protein catabolism for gluconeogenesis.



Sources of energy in late fasting states (mainly by gluconeogenesis).

H. Summary of starvation:

1. The metabolic changes that occur during starvation ensure that all tissues have an adequate supply of fuel molecules.
2. the following diagram summarizes the inter tissue relationship during starvation.



Chapter 5 *Cancer, Oncogenes, Tumor Markers and apoptosis*

I. *Introduction:*

A. **Cancer cells**: are characterized by 3 properties:

1. Diminished control of growth.
2. Invasion of local tissues.
3. Spread (metastasis) to the other parts of the body.

B. **Benign tumor cells**: are also characterized by 3 properties:

1. Diminished control of growth.
2. Do not invade local tissues.
3. Do not spread to the other parts of the body.

C. Cancer is the second most common cause of death in the USA after cardio vascular diseases. In Egypt, no statistical data about this issue is available.

II. *Causes of cancer:* Cancer may be caused by:

a) **Agents**: Radiant energy, chemical compounds, and viruses.
b) **Oncogenes**: which are genes capable of causing cancer.

A. Agents that cause cancer:

1. Radiant energy (Radiation) :

a) Ultraviolet rays, x-rays and γ-rays.

b) They cause cancer through:

1) Direct effects on DNA →DNA damage → Cancer formation.

2) Formation of free radicals e.g. superoxide → DNA damage → Cancer formation.

2. Chemicals:

About 80% of human cancer are caused by environmental factors, principally chemicals. Exposure to such compounds can occur by:

a) **Pollution.**

b) **Occupation:** e.g. exposure to benzene, asbestos.

c) **Diet:** Aflatoxin B1, which is produced by the mold *Aspergillus flavus* and sometimes food.

d) **Life style** e.g. cigarette smoking.

B. **Oncogenes:**

Human genome contains two classes of genes:

1. **Oncogenes:** which are genes that ***promote*** development of cancer (tumors).
2. **Tumor suppressor gene**: which are genes that ***suppress*** the development of cancer (Tumors).

Oncogenes

I. *Definitions:*

A. Proto-oncogenes (C-oncogenes):

These are normal genes (about 100) present in human genome. They have specific functions in cell growth and differentiation.

B. Oncogenes:

These are abnormal genes. They are mutant (altered) proto-oncogenes. They can lead to malignant tumors.

II. *Mechanisms of transformation of proto-oncogenes into oncogenes:* This can be done by 5 mechanisms:

A. Promotor insertion:

When certain viruses infect cells, a viral DNA copy (cDNA) is synthesized by reverse transcriptase and this cDNA is integrated in the host genome. These inserted new cDNA act as promoter of transcription of pro-oncogene → oncogene.

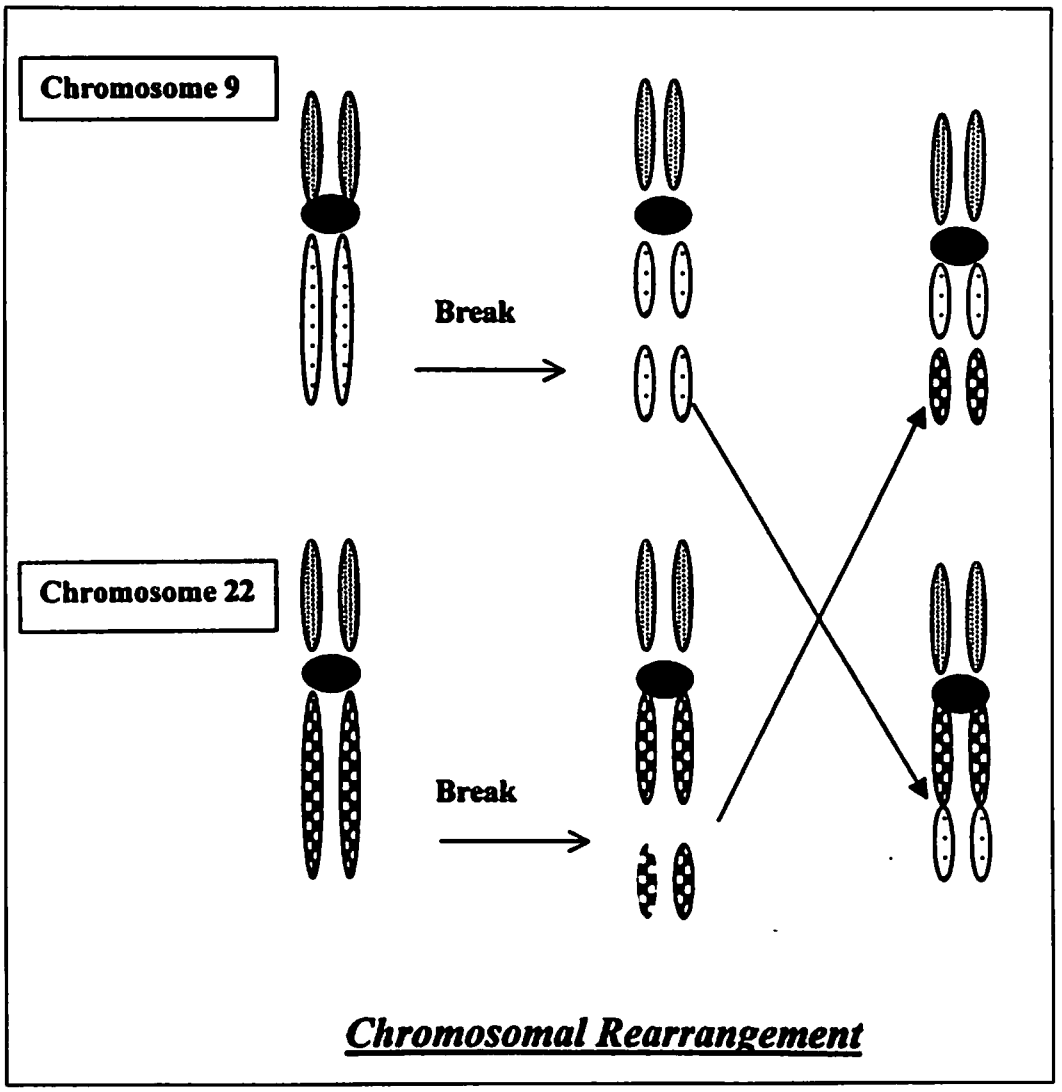
B. Enhancer insertion:

Here the viruses that infect cells produce cDNA that act as enhancer (stimulator), which stimulates DNA for transcription.

C. Chromosomal translocations: (Chromosomal rearrangements):

1. A piece of one chromosome is splitted off and then joined to another chromosome.
2. If the second chromosome donates material to the first, the translocation is said to be reciprocal.
3. **Example of chromosomal translocation:**
   a) Philadelphia chromosome (involving chromosomes 9 and 22) → chronic granulocyclic leukemia.

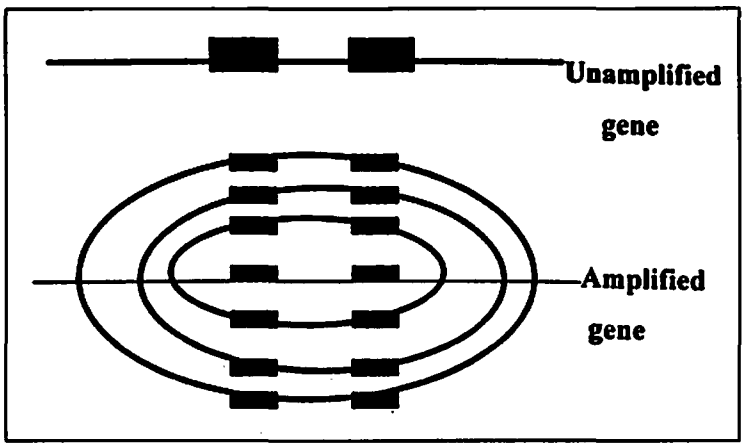
b) Chromosomal translocation of chromosomes 8 and 14 → cancer of human B-lymphocytes → Burkett's lymphoma.



D. **Gene amplification:**

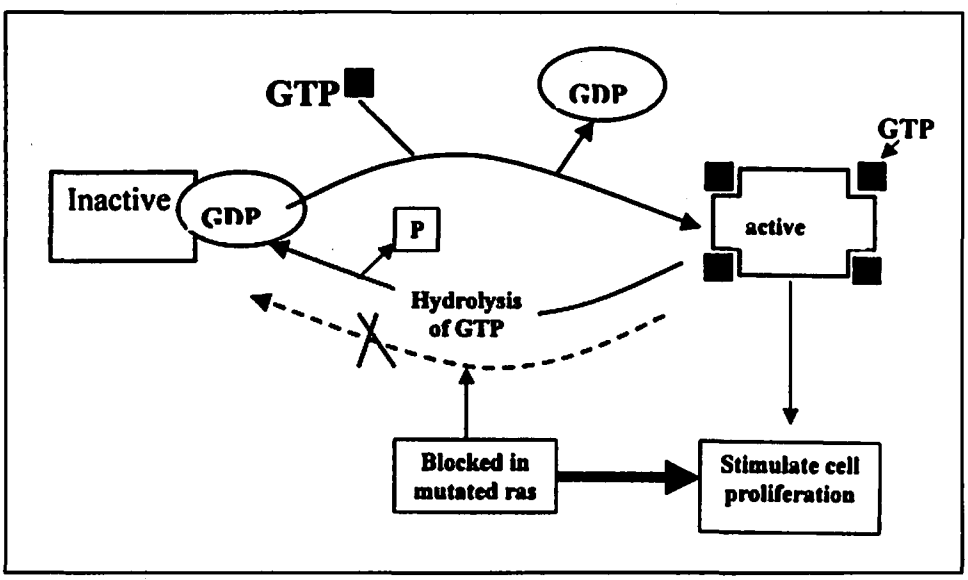
1. **This is an increase in number of copies of normal proto-oncogene within the cell e.g. genes for certain enzymes e.g. dihydrofolate reductase.**
2. **Amplification results in:**
   a) An increase of enzyme activity → Resistance to certain anti-malignant drugs as methotrexate.
   b) Amplification may play a role in the progression of tumor cells to a more malignant state.
   c) Such amplification may produce several hundred copies of the protooncogenes in the tumor cell e.g. oncogene myc in neuroblastoma and oncogene c-erb B2 in breast cancer. Each of amplified copies → Proteins → Alter cell growth → Cancer development.

of amplified copies → Proteins → Alter cell growth → Cancer development.



E. **Single point mutation:**

Sometimes a single point mutation of proto-oncogene in human cells leads to production of mutant protein → Affects G-protein in cell membrane → Affect adenylyl cyclase → affect hormonal action → **cancer:**



1. C-ras proto-oncogene from normal human cells and c-ras oncogene from a cancer of human bladder showed that they differed only in one base → Amino acid substitution at position 12 of the product protein → This change affects protein conformation and diminishes its activity as GTPase.
2. The lowered activity of GTPase results in chronic stimulation of the activity of adenylyl cyclase, which leads to a number of effects on cellular metabolism due to increased amount of cAMP.

III. *Mechanisms of action of oncogenes:*

A. They may act on key intracellular pathways involved in growth control e.g. single point mutation may result in a protein that affect mitosis.

B. The product of oncogenes may imitate the action of a polypeptide growth factor.

C. The product of oncogenes may also imitate an occupied receptor for growth factor.

IV. *Functions of some proto-oncogenes:*

A. **Synthesis of protein kinase enzyme**: One type of proto-oncogenes called The c-SRC proto-oncogenes codes for synthesis of cytoplasmic protein kinase. This enzyme helps phosphorylation of some proteins changing its activity.

B. **Formation of growth factors:** Some proto-oncogenes code for synthesis of proteins in the form of **growth factors** and growth factor receptors.

C. **Regulation of gene expression**: Some proto-oncogenes (c-Jun proto-oncogenes) act as regulator genes that regulate **gene expression**.

D. **Synthesis of protein that binds GTP**: Some proto-oncogenes (c-ras-proto-oncogenes) code for synthesis of protein that binds GTP. GTP acts as first messenger for some hormones.

V. *Growth factors:*

A. Growth factors are polypeptides exert a mitogenic response on their target cells.

B. They affect many different types of cells e.g. blood cells, nervous system, mesenchymal tissues and epithelial tissues.

C. **Growth factors act in an endocrine, paracrine or autocrine manner?**

1. **Endocrine manner:** like hormones, they may be synthesized elsewhere in the body and pass in circulation to their target cells.
2. **Paracrine manner**: they may be synthesized in certain cells and secreted from them to affect neighboring cells. However the cells that synthesized the growth factors are not themselves affected, because they lack suitable receptors.

3. **Autocrine manner**: cells that synthesize growth factors have receptors for them or they are secreted inside cells and directly stimulate various processes.

D. **Mode of action of growth factors:**

Growth factors bind with specific cell receptors on the plasma membrane of target cells forming growth factor receptor complex. This causes:

1. Phosphorylation of target proteins in the cytoplasm.
2. The growth factor- receptor complexes are subjected to endocytosis in coated vesicle (like LDL) ➔ rapid activation of certain cellular proto-oncogene ➔ Oncogenes.

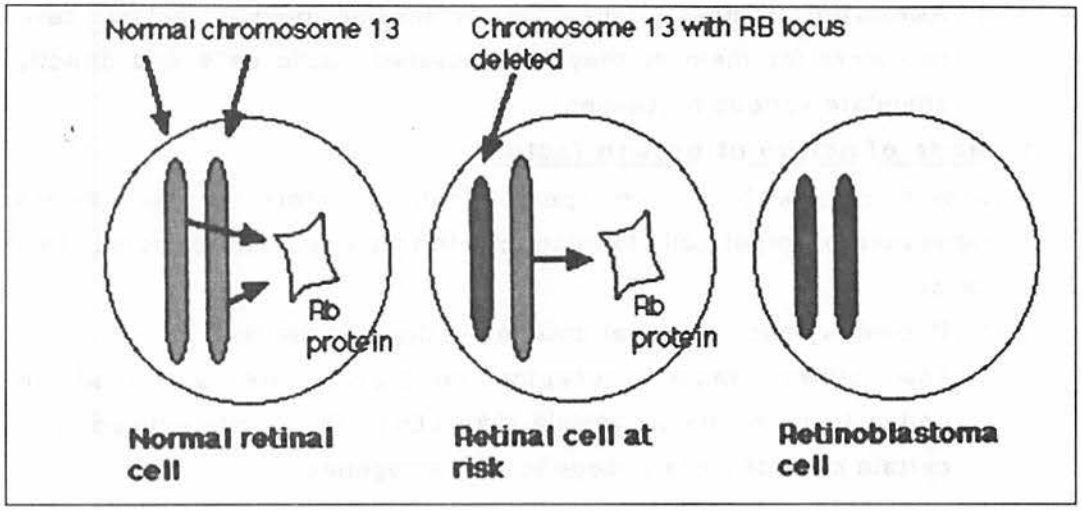
VI. *Tumor suppressor genes (anti-oncogenes):*

A. **Definition:** these are genes that suppress cancer (tumor) formation.

1. Their protein product inhibits mitosis.
2. These genes have a recessive effect at the cell level i.e. one normal allele is sufficient to prevent tumor formation even if the second allele has been inactivated or lost.
3. Oncogenes, by contrast, behave as dominants i.e. one defective allele can predispose the cell to tumor formation even if the second allele is normal.

B. **Examples of tumor suppressor genes:**

1. **RB gene (retinoblastoma gene):**
   a) Retinoblastoma is a cancerous tumor of the retina. It occurs in two forms:
      1) **Familial retinoblastoma**: Multiple tumors in the retinas of both eyes occurring in the first weeks of infancy.
      2) **Sporadic retinoblastoma**: Single tumor appears in one eye sometimes in early childhood before the retina is fully developed.
   b) **Causes of retinoblastoma:**
      1) **Familial retinoblastoma:**
         Occurs when the fetus inherits from one of its parents a chromosome (number 13) that contains a deleted or mutated RB gene. Then after birth, mutation of the remaining RB gene (somatic mutation) will remove the inhibition provided by RB protein ($p110^{RB1}$) → Retinoblastoma.



2) **Sporadic Retinoblastoma:**
In this disease, both inherited RB genes are normal but later in life after birth, both genes undergo somatic mutation (often a deletion) → Retinoblastoma.

c) In both forms of the disease, the patient's life can be saved if the tumor(s) is detected soon enough and the affected eye(s) removed.

2. **P^{53} gene:**
   a) It is a tumor suppressor gene. Its protein product is 53 kilodaltons (hence the name).
   b) **Site:** short arm of chromosome 17 in somatic and gametogenic cells.
   c) It prevents the formation of some tumors. Its mutation may lead to:
      1) Cancer lung.
      2) Cancer breast.
      3) Cancer colon.
3. **Mechanism of action of p^{53} protein:**
   a) **Regulation of cell division:** P^{53} acts as activator for transcription, regulating certain genes involved in cell division.
   b) **Control of DNA damage and repair:** If excess damage to DNA has occurred → increased p53 → Inhibition of cell division and allowing time for repair.
   c) **Protection against viral infection:** P^{53} binds with specific virus proteins, forming complex that inhibits viral activity.
   d) **P^{53} has a role in apoptosis:** Apoptosis is a programmed cell death controlled by specific gene. Stimulation of this gene →

Rapid death of the cells. p^{53} participate and stimulate apoptosis by unknown mechanism.

Tumor Markers

I. *INTRODUCTION:*

A. Definition:

Tumor markers are biologic substances synthesized and released by cancer cells; or produced by the host cells in response to the presence of cancerous tissue.

B. Site:

Tumor markers may be present in circulation, in body fluids or associated with cells: In the cytoplasm or on cell membrane.

C. Structure:

Tumor markers may be enzymes, hormones, and proteins (tumor antigen).

II. *Clinical importance of ideal tumor marker:*

Ideally, tumor marker should provide the following uses in patients having cancer:

A. **Detection of tumors**.

B. **Screening:** the asymptomatic population.

C. **Diagnosis:** the symptomatic patients, and differentiating malignant from benign conditions.

D. **Staging:** the disease, by defining extent of the diseases.

E. **Monitoring:** the response of the therapy.

F. **Assessing prognosis.**

G. **Detecting recurrence:** Early detection of disease recurrence.

III. *Properties of ideal tumor markers:*

A. Have high disease **sensitivity** i.e. it should be positive in all patients with particular cancer.

B. Have high disease **specificity** i.e. it should be negative in all normal population.

C. Its level reflects the **stage of the disease.**

D. Its level must be **stable** i.e. not subjected to marked fluctuation in stable disease state.

E. **Organ specific** i.e. positive only in certain organ tumor.

Unfortunately, no tumor marker available can fulfill all these criteria for ideal marker.

IV. *Types of tumor markers:*

They are divided into 2 types cellular and humoral.

A. Cellular (tissue) tumor markers:

They include antigens located on the cell membrane or intra cellular components as oncogenes.

B. Humoral (serum) tumor markers:

1. These are substances, which can be detected in serum.
2. They are usually synthesized and excreted by tumor cells or released on tumor disintegration or formed as a result of reaction of the organism to a tumor.

V. *Classification of tumor markers:*

Tumor markers can be classified into hormones, enzymes and tumor antigens:

A. Hormones:

1. Example of hormones that are used as tumor markers are: ATCH, ADH, calcitonin, HCG, PTH, growth hormone and prolactin.
2. The production of hormones in cancer involves two separate routes:
   a) An excess production of a hormone by the endocrine tissue that normally produces it.
   b) A hormone may be produced at a distant site by a non-endocrine tissue that normally does not produce the hormone. This condition is called "entopic syndrome".

B. Enzymes:

1. An increase in an enzyme or isoenzymes is not specific or sensitive enough to be used for identifying the type of cancer or the specific organ involvement.
2. Example of enzymes that are used as tumor markers are: alkaline phosphatase and prostatic acid phosphatase (PAP).

C. Tumor antigens:

1. Oncofetal antigens:

a) These are proteins produced normally during fetal life. They are present in high concentration in the sera of fetuses and decrease to low levels or disappear after birth.

b) In cancer patients, these proteins re-appear.

c) The production of these proteins demonstrates that certain genes are reactivated as the result of the malignant transformation of cells.

d) They include carcinoemryonic antigen (CEA) and α-fetoprotein (AFP).

2. Other tumor antigens:

a) Carbohydrate antigen 19.9 (CA 19.9).

b) Cancer antigen 125 (CA125).

c) Cancer antigen 15.3 (CA 15.3).

d) Cancer antigen 50 (CA 50).

e) Cancer antigen 27.4 (CA 27.4).

f) Squamous carcinoma antigen (SCCA).

g) Prostatic specific antigen (PSA).

h) Tissue polypeptide antigen (TPA).

3. Proteins:

a) B2 macroglobulin.

b) Ferritin.

Classification of tumor markers:

Class of markers	Examples	Tumor source
Tumor antigens	α- Fetoprotein (AFP)	**Hepatoma – teratoma**
	carcinoemryonic antigen (CEA)	**Cancer of breast, colon, lung.**
	Cancer antigen 15.3 (CA 15.3)	**Breast cancer**
	Cancer antigen 125 (CA 125)	**Ovarian cancer**
	Cancer antigen 19.9 (CA 19.9)	**Pancreatic cancer**
	Cancer antigen 72.4 (CA 72.4)	**Gastric cancer**
	Cancer antigen 50 (CA 50)	**Pancreatic carcinoma**
	Cancer antigen 549 (CA 549)	**Breast carcinoma**
	Squamous carcinoma antigen	**Cervix of the uterus**
	Prostatic specific antigen (PSA)	**Prostate cancer**
	Tissue polypeptide antigen (TPA)	**Breast and bladder cancer**
	MCA	**Breast – Ovarian**
Hormones	**ACTH**	**Carcinoma of lung, colon, prostate and ovary**
	Calcitonin	**Medullary carcinoma of thyroid**
	Prolactin	**Cancer lung**
	Parathyroid hormone (PTH)	**Parathyroid tumors**

Enzymes	**Prostatic acid phosphatase (PAP)**	**Prostatic carcinoma**
	Alkaline phosphatase	**Cancer liver & GIT**
	Neuron specific enolase (NSE)	**Small cell bronchial carcinoma and neuroblastoma**
Proteins	**B2- Macroglobulin**	**Lymphoma and multiple myeloma**
	Ferritin	**wide variety of tumors, e.g. human breast cancer and renal cell carcinoma**

VI. *Examples of some clinically important tumor markers*:

A. AFP (alpha feto protein):

1. This is the major serum protein in fetus.
2. It is synthesized by yolk sac, liver and GIT.
3. AFP level increases in cancers as cancer testes and hepatic carcinoma.

B. CEA (carcino embryonic antigen) :

1. A glycoprotein synthesized by tumor cells and normal colonic epithelium.
2. It is carried on the cell surface membrane and normally sheds with feces.
3. In cancer it sheds in serous fluids.
4. ***Raised level is non specific:***
   a) It is detected in 65% of colorectal cancer i.e. cancer of colon and rectum.

C. PSA (Prostatic specific antigen) :

1. Widely accepted tumor marker in prostatic cancer.
2. Glycoprotein produced only by prostatic epithelial cells and it is organ specific.
3. ***Normal level:*** 0-4 ng/ml
4. ***Elevated Level (> 4ng/ml) occurs in :***
   a) 65% of localized prostatic cancer.
   b) 40 % of benign prostatic hypertrophy.

D. Thyroid : (Calcitonin):

1. First degree relatives of patients with medullary thyroid carcinoma can be screened by measuring calcitonin levels (20% of these carcinomas have a familial history).

E. HCG (Human chorionic gonadotropin):

1. Produced by placenta, and used for detection of pregnancy..
2. Reaching maximum level at 8^{th} week of gestation.
3. It is produced also by abnormal trophoblasic tissue.
4. it is composed of alpha nonspecific and beta specific subunits.

5. It increases in chorion carcinoma and can detect a tumor mass of 1 mg.

Tumor markers guide:

Breast	**Prostate**
CA 15.3 CEA	PSA PAP
Ovary	**Uterus**
CA 125 CEA HCG AFP	CEA CA 125
Cervix uteri	**Testes**
CEA SCC-A	AFP HCG
Liver	**Stomach**
AFP	CA 72.4 CEA
Pancreas	**Urinary bladder**
CA 19.9 CEA AFP	TPA CEA
Lung	**Thyroid**
CEA NSE	Calcitonin CEA Thyroglobulin
Colorectum	**Leukemia**
CEA CA 19.9	β2M LDH
Lymphoma	**Multiple meyloma**
β2 M LDH	β2 M

Apoptosis

For every cell, there is a time to live and a time to die

I. *Definitions:*

A. Apoptosis is a Greek word means: [**apo** = away from + **ptosis** = fall].

B. Apoptosis is a programmed cell death or "cell suicide". It is the body's normal method of ending the life cycle of cells through the cellular self-destruction.

C. Apoptosis leads to the elimination of cells without releasing harmful substances into the surrounding area.

D. The cell shrinks, dissolves its contents, and activates phagocytosis by neighboring cells.

E. ***If apoptosis is affected, then the cell will not die, causing a malignant condition.***

II. *Importance of apoptosis:*

A. **The formation of the fingers and toes of the fetus** requires the removal, by apoptosis, of the tissue between them.

B. **The sloughing off of the inner lining of the uterus** (the endometrium) at the start of menstruation occurs by apoptosis.

C. Programmed cell death is needed **to destroy cells that represent a threat to the integrity of the organism.**

D. **Cells infected with viruses (e.g. AIDS).** One of the methods by which c lymphocytes kill virus-infected cells is by inducing apoptosis.

III. *The Mechanisms of Apoptosis:* There are 3 different mechanisms by which a cell commits suicide by apoptosis:

A. **Through binding of death activators to receptors at the cell surface e.g. tissue necrosis factor (TNF-α).**

B. **Through triggering by dangerous reactive oxygen species.**

C. **Through signals arising within the cell.**

1. Upon receiving specific signals instructing the cells to undergo apoptosis,a number of distinctive changes occur in the cell.

2. A family of proteins known as **caspases** are typically activated in the early stages of apoptosis. These proteins breakdown or cleave key cellular components that are required for normal cellular function including structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes.

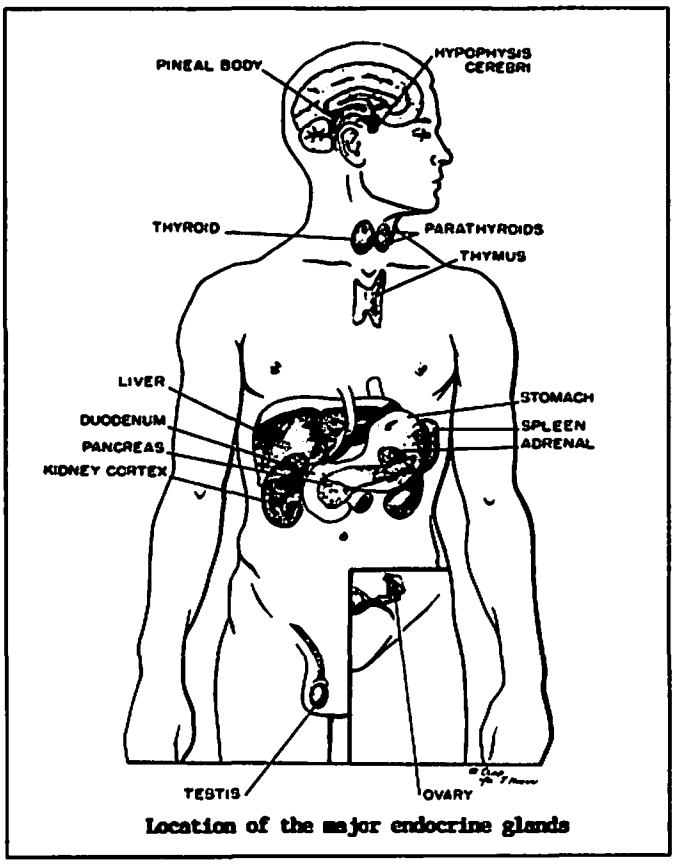
3. The caspases can also activate other degradative enzymes such as DNases, which begin to cleave the DNA in the nucleus.

Chapter 6 *Hormones*

I. *Definition:*

A. **Hormones** are organic compounds produced by the endocrine system and secreted directly into the blood to act near to their site of release or at a distant organ in the body.

B. **The endocrine system** is all the hormones producing tissues.



Location of the major endocrine glands

II. *General functions of hormones:*

A. **Regulation of metabolism**: Hormones affect the metabolism of carbohydrate, protein, lipids and minerals, directing their synthesis, storage, mobilization and utilization according to needs.

B. **Growth**: The growth of bones, viscera and various types of tissues is under the control of hormones.

C. **Homeostasis**: Hormones help the maintenance of internal environment.

D. **Behaviour**: Hormones have an important role in behaviour. Fear, depression and sex behaviour are due to several natural hormonal factors.

E. **Reproduction**: Reproductive organs are highly sensitive to hormones.

III. *Classification of hormones:*

Hormones are classified according to chemical composition, solubility properties and mechanism of action.

Classification of hormones by mechanism of action.

Group I. Hormones that bind to intracellular receptors	
Estrogens	Calcitriol (1,25$[OH]_2$-D_3)
Glucocorticoids	Androgens
Mineralocorticoids	Thyroid hormones (T_3 and T_4)
Progestins	
Group II. Hormones that bind to cell surface receptors	
A. The second messenger is cAMP.	
Adrenocorticotropic hormone (ACTH)	Parathyroid hormone (PTH)
Angiotensin II	Opioids
Antidiuretic hormone (ADH)	Acetylcholine
Follicle-stimulating hormone (FSH)	Glucagon
Human chorionic gonadotropin (hCG)	α_2-Adrenergic catecholamines
Lipotropin (LPH)	Corticotropin-releasing hormone (CRH)
Luteinizing hormone (LH)	Calcitonin
Melanocyte-stimulating hormone (MSH)	Somatostatin
Thyroid-stimulating hormone (TSH)	β-Adrenergic catecholamines
B. The second messenger is cGMP.	
Atrial natriuretic factor (ANF)	
C. The second messenger is calcium or phosphatidylinositides (or both):	
α_1-Adrenergic catecholamines	Acetylcholine (muscarinic)
Cholecystokinin	Oxytocin
Gastrin	Gonadotropin-releasing hormone (GnRH)
Substance P	Angiotensin II
Thyrotropin-releasing hormone (TRH)	
Vasopressin	
D. The intracellular messenger is unknown:	
Chononic somatomammotropin (CS)	
Growth hormone (GH)	Nerve growth factor (NGF)
Insulin	Epidermal growth factor (EGF)
Insulinlike growth factors (IGF-I, IGF-II)	Fibroblast growth factor (FGF)
Prolactin (PRL)	Platelet-derived growth factor

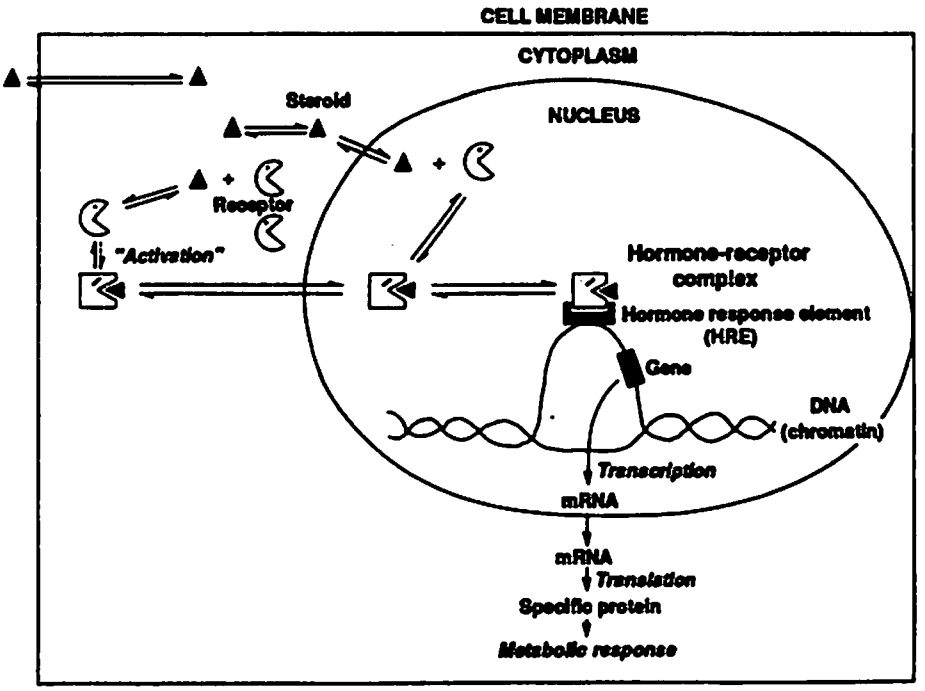
A. **Classification according to the chemical composition:**
   1. **Amino acid derivatives:** Such as thyroid hormones (T_3, T_4), epinephrine, nor epinephrine and serotonin.
   2. **Polypeptides:** Such as pituitary hormones e.g. oxytocin, ADH, ACTH.
   3. **Proteins and glycoproteins:** Such as insulin, growth hormone, TSH, LH, FSH, parathyroid hormone, prolactin etc.
   4. **Steroids:** Such as adrenal cortical hormones, sex hormones: estrogens, progesterone and androgens.

B. **Classification according to the mechanism of action:** There are 2 groups:
   1. **Group I:** Includes hormones that bind to the intracellular receptors.
   2. **Group II:** Includes hormones that bind to cell surface receptors.
   3. **Characters of group I and II:**

	Group I	Group II
Types	Steroids T3-T4 and catecholamines	Amino acid derivatives, polypeptides,, proteins and glycoproteins.
Solubility	Hydrophobic	Hydrophilic
Plasma transport protein	Present (to help solubility and transport in plasma)	Absent
Plasma half life	Minutes and hours	Short (minutes)
Receptors	Intracellular	Present in cell Membrane
Mediator	Receptor-hormone complex	cAMP, calcium and other

C. **Mechanism of action of group I: Hormones that bind intracellular receptors :**
   1. The hormone diffuses through the cell membrane of the target cells and binds to specific receptors protein in the cytosol or the nucleus, forming a complex.
   2. The hormone-receptor complex then undergoes activation reaction, which leads to change in size, conformation and surface charge of this complex, making it able to bind to DNA at specific region called "hormone response element "(HRE).
   3. The hormone-receptor complex in this position will activate or inactivate the promoter element; PE (which is a part of DNA that determines the initiation of the transcription). This will affect the transcription of specific genes, and production of mRNA and protein molecules.

4. **Therefore, the amount of specific proteins is changed (increased or decreased) and metabolic process is influenced.**



Mechanism of action of group I hormones: (binding with intracellular receptors).

D. **<u>Mechanism of action of group II:</u> Hormones that bind to cell surface receptors:**

1. **A hormone (first messenger) binds to a specific receptor located in the cell membrane to activate a second messenger which is located in the cytosol.**
2. **The second messenger may be one of the following molecules cyclic AMP (cAMP), cyclic GMP (cGMP), calcium or phospholipids.**

Subclassification of group II. A hormones.

Hormones That Stimulate Adenylate Cyclase (H_s)	Hormones That Inhibit Adenylate Cyclase (H_i)
ACTH	Acetylcholine
ADH	α_2-Adrenergics
βAdrenergics	Angiotensin II
Calcitonin	Opioids
CRH	Somatostatin
FSH	
Glucagon	
hCG	
LH	
LPH	
MSH	
PTH	
TSH	

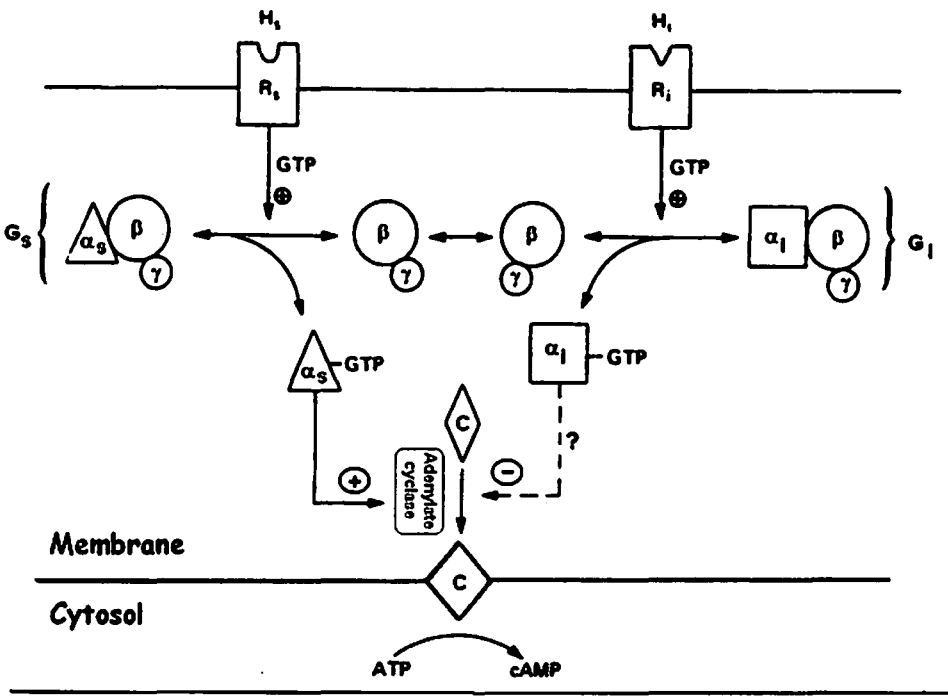
3. **Hormones use cAMP as the second messenger:**
   a) **These hormones are subclassified into:**
      1) **Hormones that stimulate adenylate cyclase and promote cAMP formation.**
      2) **Hormones that inhibit adenylate cyclase and stop cAMP formation.**

***b)* Hormones that stimulate adenylate cyclase do so by:**

1) Hormone binds to cell membrane receptors which are called **stimulatory receptors (R_s)**, forming a hormone receptor complex.
2) This complex in the presence of GTP will activate another regulatory protein, which is called **stimulatory protein (G_s)**.
3) This stimulatory protein (G_s) is composed of 3 subunits, α, β and γ , and the reaction occurs as follows:

$$\text{Regulatory protein } (\alpha, \beta, \gamma) \underset{\text{GTPase}}{\overset{\text{GTP}}{\rightleftharpoons}} \text{active regulatory protein } (\alpha_s\text{-GTP}) + \beta\gamma$$

4) α_s - GTP complex will stimulate adenylate cyclase enzyme present at the inner surface of the cell membrane.
5) Then active adenylate cyclase will convert ATP into cAMP (second messenger) in the cytosol.
6) Cyclic AMP will stimulate an enzyme called protein kinase. This enzyme is composed of 4 subunits: 2 regulators (R) and 2 catalytic (C). The whole protein kinase is inactive, but binding of cAMP with the enzyme dissociates R from C and activating it.



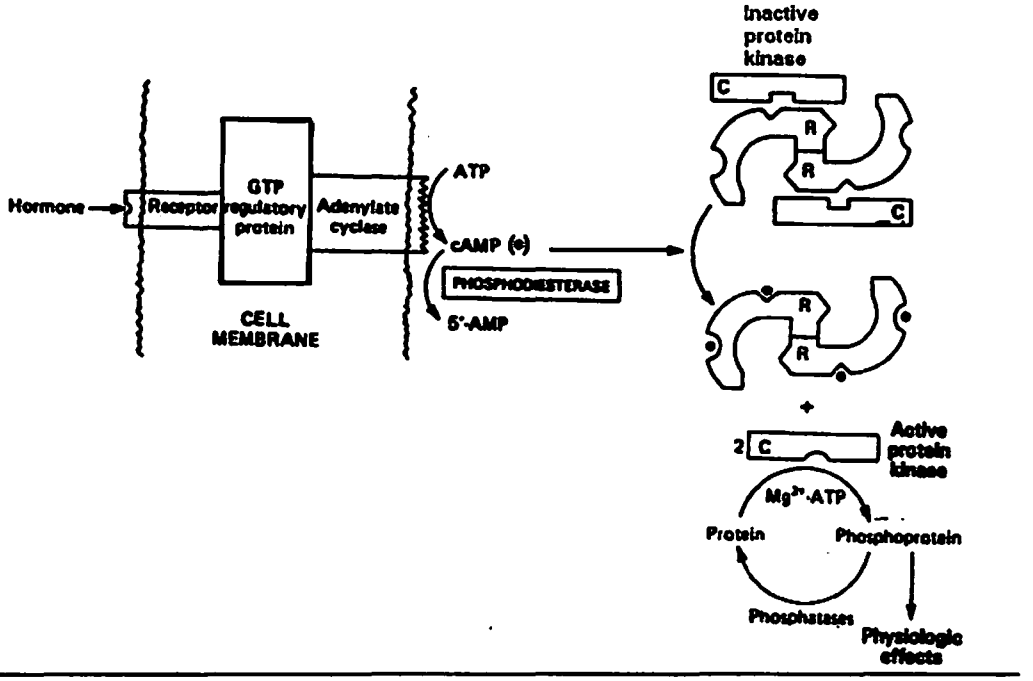
Mechanism of action of hormones that stimulate adenylate cyclase.

7) The active C subunits (active protein kinase)catalyses

the transfer of phosphate group (phosphorylation) from ATP to many enzyme:

i- Some phosphorylated enzymes become active e.g. phosphorylase, hormone sensitive triacylglycerol lipase.

ii- Some other phosphorylated enzymes become inactive e.g. glycogen synthase.



Homones that stimulate adenylate cyclase.

c) Hormones that inhibit adenylate cyclase do so by the same previous mechanism using:

1) Cell membrane inhibiting receptors (R_s).
2) Regulatory inhibiting protein (C_i).
3) The resulting α_i - GTP will inhibit activation of adenylate cyclase (see the figure).

4. Hormones use cGMP as the second messenger:

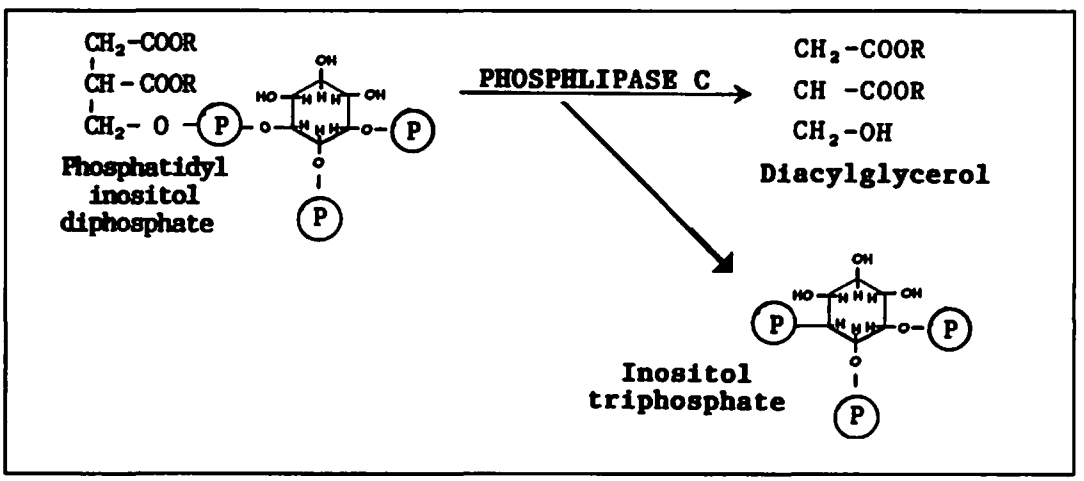
a) There is only one hormone uses cyclic GMP as a second messenger which is atrial natriuretic factor (ANF), produced by arteries of the heart.

b) Mechanism of action :

1) ANF (first messenger) binds to the cell membrane receptors, forming a hormone-receptor complex.
2) This complex activates guanylate cyclase enzyme present in the cell membrane, which in turn converts GTP into cGMP (second messenger) in the cytosol.

3) cGMP will activate cGMP-dependent protein kinase which in turn phosphorylates a number of proteins causing the functions of ANF to start, which are:
   - i- Relaxation of smooth muscles and vasodilatation.
   - ii- Excess excretion of sodium (natriuresis).
   - iii- Excess excretion of water (diuresis).

5. **Hormones use calcium and phospholipids as a second messenger :**
   - *a)* Hormone (first messenger) binds with the receptor forming hormone-receptor complex.
   - *b)* This complex, in the presence of **G protein** will activate phospholipase c enzymes.
   - *c)* **Active phospholipase c will cause :**
     1) Stimulation of calcium entry (influx) through the cell membrane (by increasing Na^+/Ca^{++} and H^+/ Ca^{++} exchange).
     2) Conversion of **phosphatidyl inositol diphosphate** (PIP_2) into diacylglycerol and inositol triphosphate:



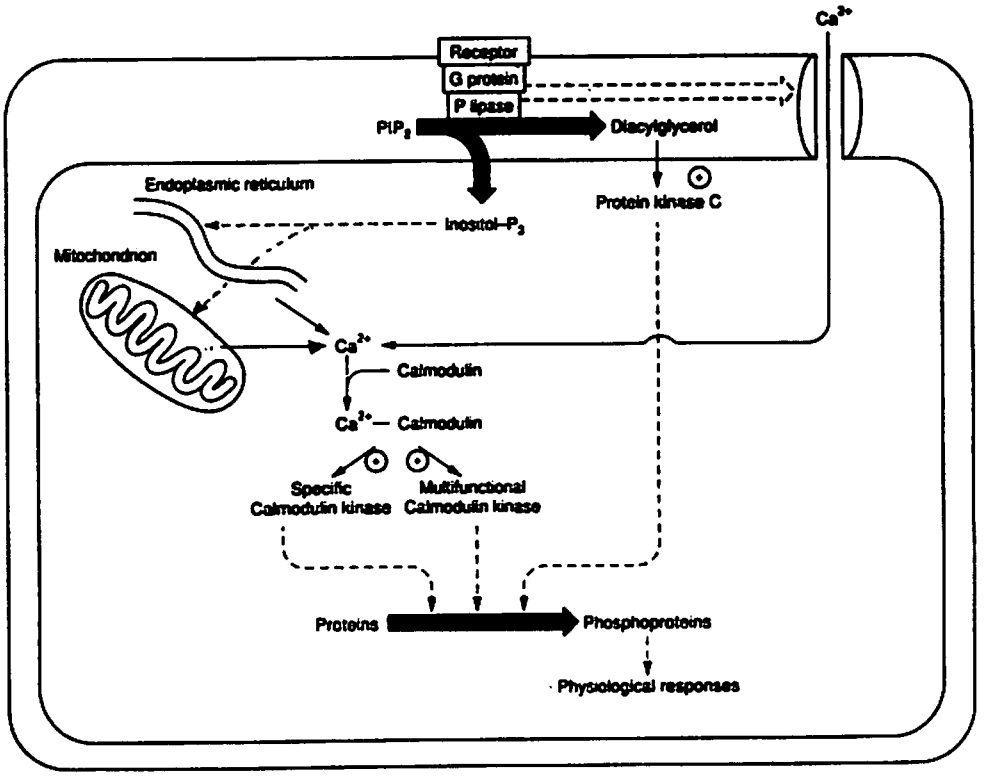
- i- **Diacylglycerol** in the presence of Ca^{++} will stimulate protein kinase c enzyme which in turn phosphorylates and activates some enzymes.
- ii- **Inositol triphosphate** will increase calcium release from cellular organelles and mitochondria and endoplasmic reticulum :
  - The released calcium will activate specific protein called: calmodulin.
  - Active calmodulin will stimulate specific calmodulin kinase and multifunctional calmodulin kinase which in turn phosphorylate and activate some proteins (enzymes).

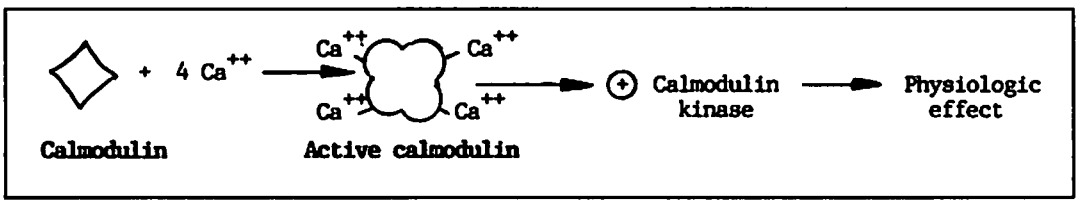
6. Calmodulin:

a) **Calmodulin is an intracellular protein of molecular weight 17000.**

b) **It is similar to the muscle protein troponin c in structure and function.**

c) **Calmodulin has 4 calcium binding sites. Full occupancy of these sites leads to marked conformational changes and activation of it.**





d) **Functions of calmodulin:**

1) **Activation of certain intracellular enzymes important for metabolism e.g. phosphorylase enzyme of glycogenolysis.**

2) **Regulation of the activity of many structural elements in the cells e.g. actinomyosin complex of smooth muscles, cell motility, mitosis, granular release and endocytosis.**

7. **Hormones with unknown intracellular messenger :**

a) A large number of important hormones have no identified intracellular messenger. Insulin is one of these hormones (see the table, group II).

Hypothalamic hormones

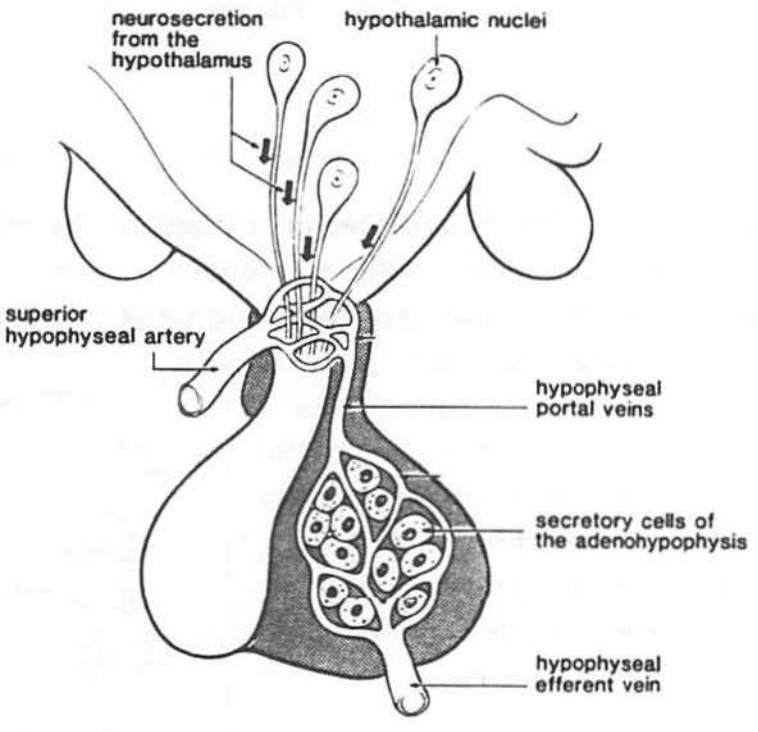
I. *Introduction:*

A. Hypothalamus is a protein of central nervous system, located at the base of the brain just above the pituitary gland.

B. Hypothalamus secretes releasing hormones, vasopressin (ADH) and oxytocin hormones.

II. *Releasing hormones :*

A. These are short peptide hormones released from hypothalamic nerve fiber endings and reach the anterior lobe of pituitary through the special portal system that connects the hypothalamus and anterior pituitary lobe.



Hypothalamic hormones.

B. Hypothalamic releasing hormones:

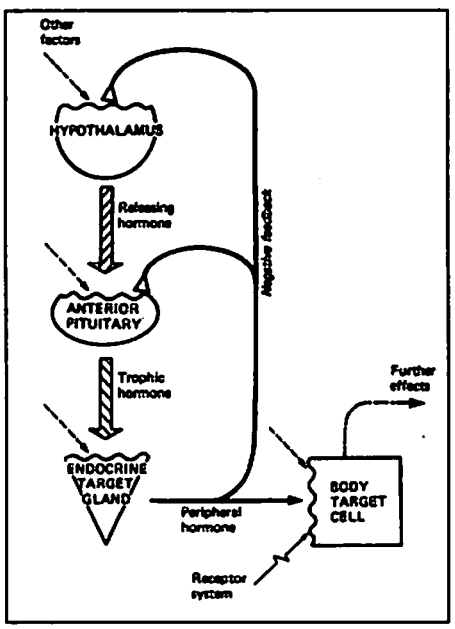
Hypothalamic hormone	Abbreviation	Pituitary Hormone affected	Target gland / hormone affected
Corticotropin releasing hormone	CRH	ACTH	Adrenal cortex / hydrocortison
Thyrotropin releasing hormone	TRH	TSH(PRL)	Thyroid gland / T_3 - T_4
Gonadotropin releasing hormone	GnRH (LHRH , FSHRH)	LH, FSH	Testis, ovary/ testosterone, estrogens and progesterone.
Growth hormone releasing hormone (stimulates GH secretion .	GHRH	Growth hormone	Liver / somatomedin c and others.
Growth hormone release-inhibiting hormone (=somatostatin) (inhibits GH secretion).	GHRIH	Growth hormone (TSH, FSH, ACTH)	
Prolactin release-inhibiting hormone (inhibits prolactin secretion).	PRIH	Prolactin	

C. **<u>The function of hypothalamic releasing hormones</u> is to regulate the secretion of anterior pituitary hormones.**

D. **<u>Hpothalamic hormones are secreted under control of</u> higher brain centers, anterior pituitary hormones and final target hormones (feed back regulation) e.g. TRH secretion is inhibited by excess concentration of TSH, T_3 and T_4.**

E. **LH and FSH are controlled by the concentration of releasing hormone: gonadotropin-releasing hormone (GnRH).**

F. **Prolactin has no releasing hormone , but it has 2 releasing inhibiting hormones:**
   1. **Dopamine.**
   2. **GAP (gonadotropin releasing**



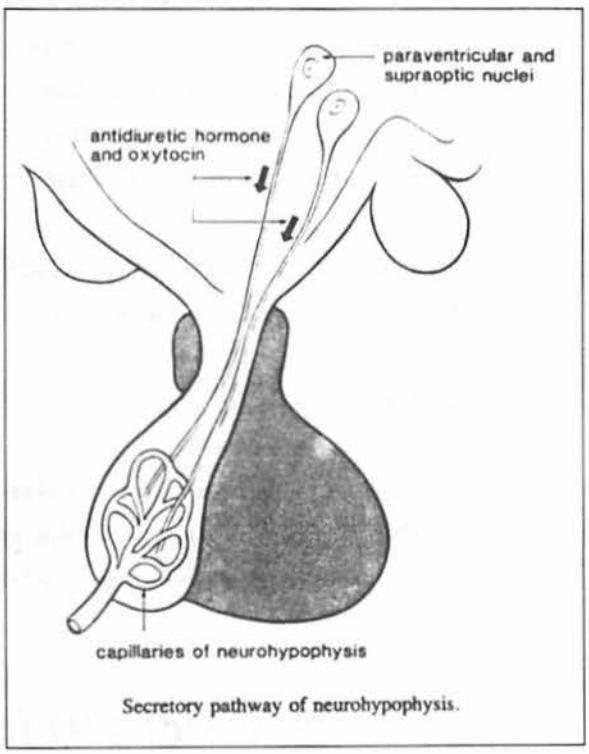
hormone associated peptide) which is found to inhibit prolactin, FSH and LH.

G. Many of hypothalamic hormones in particular TRH, CRH and somatostatin are fond in other portions of the nervous system and in a variety of peripheral tissues.

III. Antidiuretic hormone [ADH (vasopressin)] and oxytocin:

A. Precursors of these hormones are synthesized cells in the hypothalamus (paraventricular and supraoptic nuclei) and travel from the hypothalamus through axons that terminate in the posterior lobe of the pituitary.

B. Before secretion, the large precursor molecules are cleaved to give the peptide hormones: ADH and oxytocin.



Secretory pathway of neurohypophysis.

C. **Regulation of secretion of ADH:**

1. The increased osmolality of the plasma is the stimulus that activates the supraoptic nuclei and ADH secretion.
2. The increased osmolality is mediated by osmoreceptors located in the hypothalamus and by baroreceptors located in the heart and other regions of vascular system.
3. Other stimuli include emotional, physical stress and some pharmacologic agents as acetylcholine, nicotine and morphine.

D. **Mechanism of action of ADH:**

1. ADH acts on distal convoluted and collecting tubules of the kidney causing water reabsorption.

E. **Diabetes insipidus:**

1. This is a disease caused by a deficiency of ADH action .
2. It is characterized by the excretion of large volumes of dilute urine.
3. **Types of diabetes insipidus:**

a) **Primary diabetes insipidus:**

Deficient ADH is usually due to destruction of the hypothalamic-hypophyseal tract from fracture of skull base, tumour or infection.

b) **Hereditary nephrogenic diabetes insipidus:**

ADH is secreted normally, but its receptors in kidney are defective.

F. **Regulation of secretion of oxytocin:**

1. Stimulation of the nipple is the primary stimulus, which sends a neural impulse to hypothalamus to secrete oxytocin.
2. Vaginal and uterine distensions are secondary stimuli.
3. Estrogens stimulate while progesterone inhibits the production of oxytocin.

G. **Mechanism of action of oxytocin:**

1. Is unknown, but oxytocin causes contraction of uterine smooth muscles.
2. Oxytocin can be used in pharmacologic amounts to induce labour in humans.
3. Oxytocin causes contraction of myoepithelial cells surrounding the mammary alveoli. This promotes the movement of milk into the alveolar duct system and allows for milk ejection.

Anterior pituitary hormones

I. *Definitions and classification:*

A. Anterior pituitary is a part of pituitary gland, which is about 10 mm in diameter. It is located in the brain just behind the optic chiasma (as an extension from the floor of the hypothalamus). The average weight of the gland in the male is 0.5-0.6 g; in the female, it is slightly larger 0.6- 0.7 g.

B. The hormones of the anterior pituitary gland (except growth hormone and prolactin) mainly act on other target, endocrine glands to stimulate the production or release of peripheral hormones.

C. Secretion of anterior pituitary hormones is regulated by feed back regulation by the increase of the concentration of peripheral hormone either directly or by acting on the hypothalamus.

D. Classification :anterior pituitary gland secretes three hormone groups:

1. Protein hormone group : It includes:

a) Growth hormone.

b) Prolactin.

c) Placental lactogen (chorionic somatomammotropin; CS).

2. **Glycoprotein hormone group : It includes:**
   a) Thyroid stimulating hormones (TSH).
   b) Luteinizing hormone (LH).
   c) Follicle-stimulating hormone (FSH).
   d) Chorionic gonadotropin (CG).
3. **Pro-opio-melano-cortin (POMC) peptide group: It includes:**
   a) ACTH.
   b) Melanocyte stimulating hormone (MSH).
   c) β-lipoprotein.
   d) Endorphins.
   e) Enkephalin.

II. Protein hormone group :

A. Growth hormone: (GH , Somatotropin):

1. **Structure: GH is a single chain polypeptide consisting of 191 amino acids with molecular weight of about 22,000.**
2. **Human GH made by recombinate DNA techniques is now available for therapeutic use.**
3. **Actions:**
   a) **Normal growth: This is mediated by a substance called : somatomedin c which is released from the liver and has many anabolic effects.**
   b) **Protein synthesis: GH increases the transport of amino acid into muscle cells and increase protein RNA and DNA synthesis.**
   c) **Carbohydrate metabolism: GH generally antagonizes the effects of insulin , causing hyperglycemia.**
   d) **Lipid metabolism: GH promotes the release of free fatty acids and glycerol from adipose tissue . It also causes increased oxidation of free fatty acids and ketogenesis in the liver.**
   e) **Mineral metabolism: GH increases calcium , magnesium and phosphate absorption and helps their incorporation in the skeleton . Thus GH promotes growth of long bones. GH causes also retention of Na^+ , K^+ and Cl^-.**
   f) **Prolactin like effect : GH binds to lactogenic receptors and thus has many of the properties of prolactin, such as stimulation of mammary gland and milk production.**

4. **Overproduction of GH (gigantism and acromegaly):**
   a) **GH excess usually occurs as a result of acidophillic tumor.**
   b) **Gigantism: is a disease resulting from excessive production of GH before the closure of epiphyseal plates, where there is accelerated growth of the long bones.**
   c) **Acromegally : is a disease resulting from excessive production of GH after the colsure of epiphyseal plates and the cessation of long bones growth.**
5. **Underproduction of GH (Dwarfism):**
   a) **Deficiency of GH in infants leads to failure of growth and a disease called: dwarfism.**

B. **Prolactin (PRL; Lactogenic hormone, mammotropin, Luteotropic hormone, LTH):**
   1. **Structure: PRL is a protein hormone with molecular weight of about 23,000.**
   2. **Actions: PRL, in combination with estrogens and progesterone, initiates and maintains lactation.**
   3. **Galactorrhoea : (discharge of milk from the breast) in both men and women may be due to a pituitary tumor causing hyperprolactinemia , often with low gonadotropins secretion.**

C. **Chorionic somatomammotropin (CS; Placental lactogen): This hormone has no definite function in humans. It seems that its metabolic effects are similar to growth hormone.**

III. ***Glycoprotein hormone group:*** **This group includes TSH as well as the gonadotrpins (FSH , LH and hCG).**

A. **Thyroid stimulating hormone (TSH):**
   1. **It is glycoprotein formed of 2 polypeptide chains; α, β with molecular weight of 30,000.**
   2. **It acts on thyroid gland, stimulating the synthesis and release of thyroid hormones(T3, T_4).**

B. **Gonadotropins (FSH, LH and hCG):**
   1. **These hormones are responsible for gametogenesis (i.e. formation and development of ovum in females and spermatozoon in males) and steroidogenesis (i.e. formation of steroid hormones) in ovary and testis.**
   2. **Gonadotropins are glycoproteins with a molecular weight of about 25,000.**

3. The mechanism of action of all gonadotropins is through binding to cell membrane receptors, stimulating adenylate cyclase and increasing cyclic AMP.

4. ***Follicle stimulating hormone (FSH):***
   a) In females, the target cells are the follicular cells in the ovary where it causes maturation of the ovarian follicles and release of estrogens.
   b) In males, the target cells are the Sertoli cells in the testis where it stimulates spermatogenesis.
5. ***Luteinizing hormone (LH):*** also in males sometimes called interstitial cell stimulating hormone, ICSH):
   a) In females: It stimulates maturation of corpus leteum and the production of progesteron by it.
   b) In males :It stimulates the production of testosterone by the leydig cells of the testis.
6. ***Human chorionic gonadotropin (hCG):***
   a) This hormone is not formed in the anterior pituitary, but synthesized in the placenta(in syncytiotrophoblast cells).
   b) It is structurally and biologically very similar to luteinizing hormone.
   c) hCG actions are:
      1) Maintaining the functions of corpus luteum during the first weeks of pregnancy .
      2) It stimulates secretion of testosterone in fetus .
   d) Pregnancy diagnosis test :
      1) hCG blood and urine concentrations are increased few days after implantation of the ovum in the uterus .
      2) Detection of hCG in either blood or urine indicates the occurrence of pregnancy.

IV.*PRO-OPIOMELANOCORTIN (POMC) PEPTIDE GROUP:* POMC is a peptide formed of 285 amino acids. It acts as a precursor for the following hormones:

A. Adrenocorticotropic hormone (ACTH):

1. Structure: ACTH is a single chain polypeptide consisting of 39 amino acids.
2. Actions:
   a) ACTH increases the synthesis and release of adrenal steroids by enhancing the conversion of cholesterol to pregnenolone.

b) **ACTH increases adrenal cortical growth (tropic effect) by enhancing protein and RNA synthesis.**

3. **Overproduction of ACTH (Cushing's syndrome): It is manifested by:**
   a) **Hyperpigmentation :due to excess associated MSH like activity.**
   b) **Metabolic manifestation due to excessive production of adrenal steroid : which includes:**
      1) **Negative nitrogen balance.**
      2) **Deficiency of potassium and phosphrous.**
      3) **Sodium retention which can lead to hypertension.**
      4) **Oedema.**
      5) **Glucose intolerance and diabetes mellitus.**
      6) **Increased plasma fatty acids, and redistribution of body fat i.e.truncal obesity.**

B. <u>Melanocyte stimulating hormones (MSH)</u>:

1. **Three types of MSH (α, β and γ) are recognized. α-MSH is derived from ACTH, while β & γ are derived from β-lipoprotein.**
2. **Their action is to stimulate melanogenesis by causing dispersion of intracelular melanin granules, resulting in darkening of the skin.**

C. <u>β-Lipotropin (β-LPH)</u>:

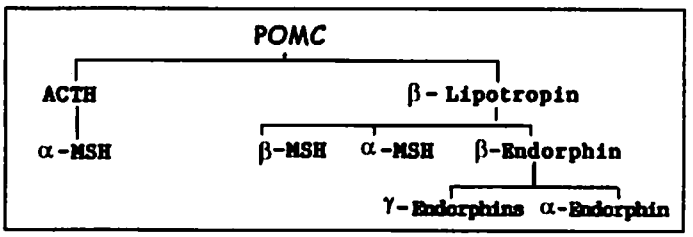
1. **β-lipotropin causes lipolysis and fatty acid mobillization.**
2. **It serves as a precursor of β-endorphins, met-enkephalin and β & γ MSH.**

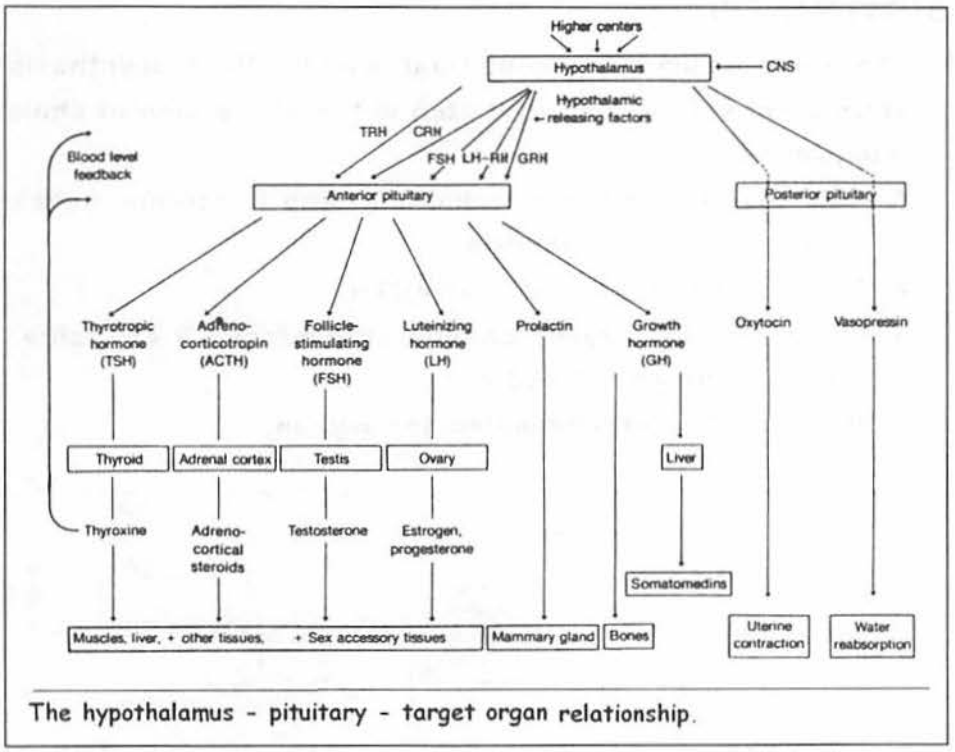
D. <u>Endorphins</u>:

1. **β-endorphins are derived from β-lipoprotein. α and γ endorphins are modifications of β-endorphins.**
2. **They act as neurotransmitters by binding to the same CNS receptors as morphin to cause pain relief and analgesia. Its analgesia power is 18-30 times more than morphine.**

E. <u>Enkephalins</u>:

1. **Enkephalins are pentapeptide forming of 5 amino acids.**
2. **Its function is like endorphins.**





The hypothalamus - pituitary - target organ relationship.

Hormones of adrenal cortex

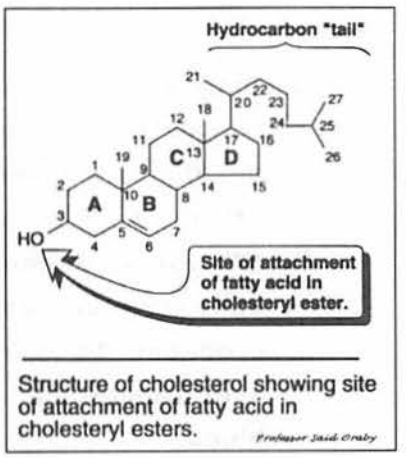
I. *CLASSIFICATION:*

A. The hormones of adrenal cortex which have a biological activity can be classified into 3 classes: glucocorticoids , mineralocorticoids and androgens.

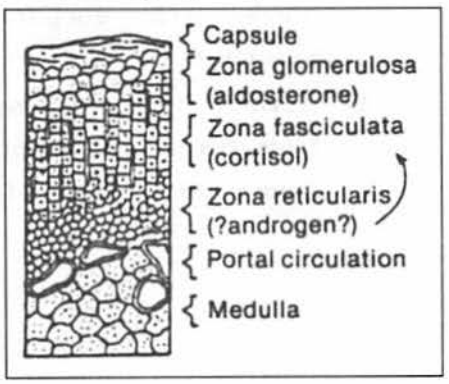
B. All contain steroid ring and derived from cholesterol.

C. Glucocorticoids and mineralocorticoids contain 21 carbon atoms and have 2 carbon side chain at C-17. Androgens contain 19 carbon atoms and have keto or hydroxyl group at C-17.

D. Glucocrticoids and androgens are synthesized in zona fasciculata and zona reticularis, while mineralocorticoids are synthesized in the subcortical zona glomerularis of adrenal cortex.



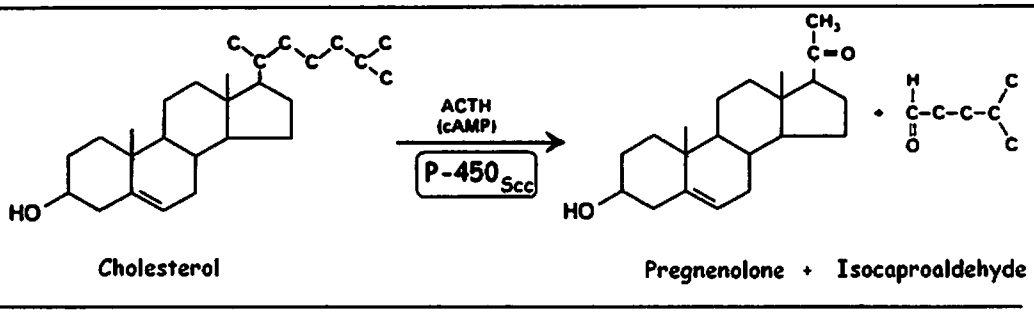
Structure of cholesterol showing site of attachment of fatty acid in cholesteryl esters.



II. *BIOSYNTHESIS:*

A. There is a common metabolic pathway for the biosynthesis of all steroid hormones. The fiirst step is the conversion of **cholesterol** into pregnenolone.

1. This reaction is the **rate limiting step** in steroidogenesis and occurs in the **mitochondria.**
2. This reaction is activated by **ACTH.**
3. It needs an enzyme called: **cytochrome P-450 side chain cleavage enzyme (P-450 scc).**
4. It requirs **NADPH** and **molecular oxygen.**



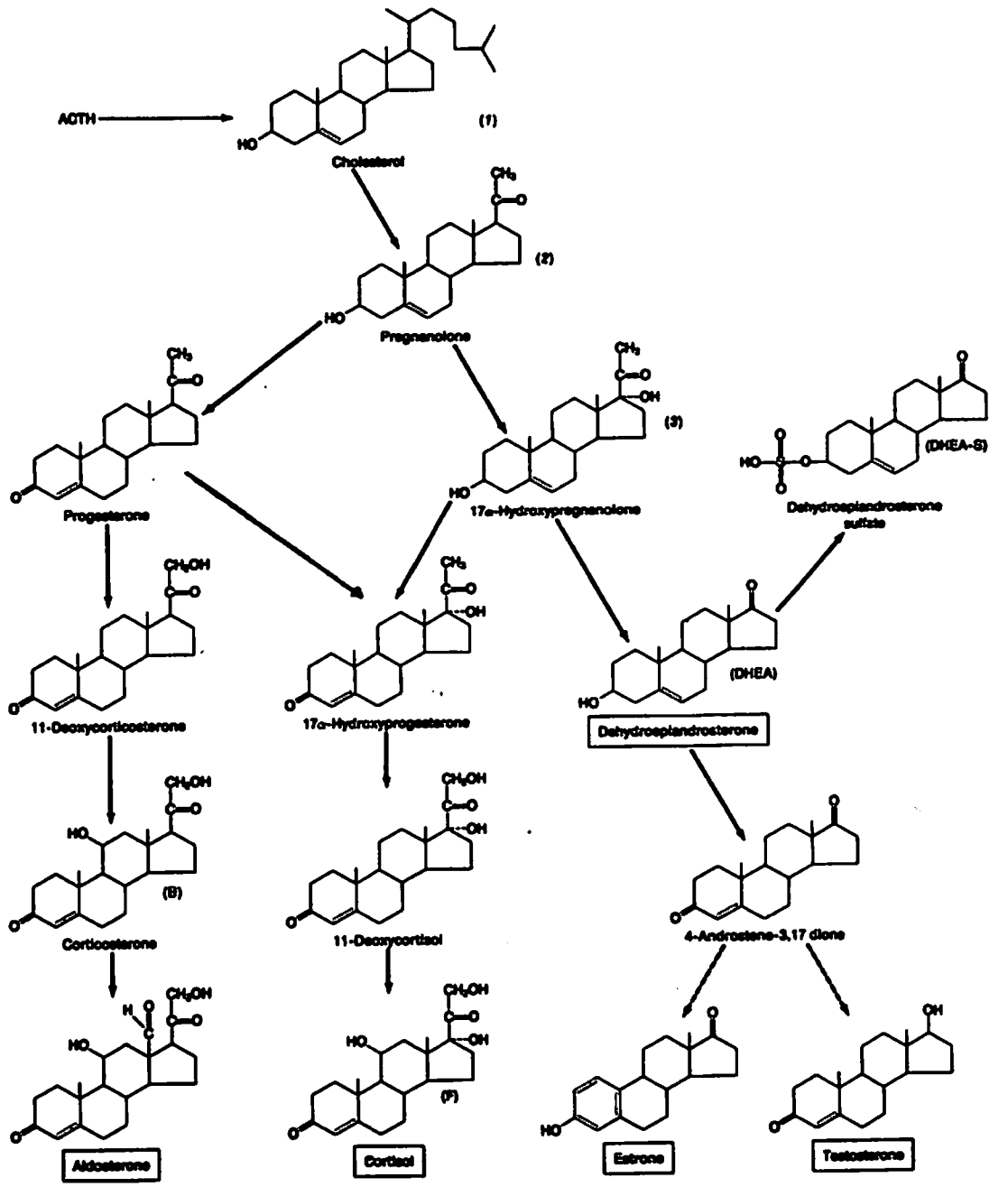
Cholestero side chain cleavage.

B. Pregnenolone is next oxidized and then isomerized to progesterone , and 17-hydroxypregnenolone, both are further modified by a series of hydroxylation and oxidation reactions to other steroid hormones.

C. 17-hydroxypregnenolone is converted into dehydroepiandrosterone (DHEA) which is the main androgen produced by adrenal cortex. DHEA is then converted by -a sulfotransferase- to DHEA sulphate, which is then secreted in the blood.

D. The major steroid hormones secreted by the human adrenal cortex. are: cortisol, corticosterone, aldosterone and DHEA sulphate.

E. Adrenal cortex can synthesize very small amount of testosterone from DHEA . Also small amount of estrogens can be synthesized from aromatization of testosterone.



Biosynthesis of steroid hormones.
Early phase of biosynthesis includes events from ACTH stimulation to rate-limiting step at (1), cholesterol side-chain cleavage to form pregnenolone. Later phases occur at key branch points (2) and (3) and additional hydroxylations to form the steroid secretory products.

III. *PLASMA TRANSPORT :*

A. Glucocorticoids:

1. Cortisol circulates in plasma in free form (8%) and in association of protein (92%).
2. The free cortisol is the biologically active form of the hormone.

3. **The cortisol binding protein is called: transcortin or corticosteroid binding globulin (CBG).**
4. **Very small amount of cortisol is bound to albumin.**

B. **<u>Mineralocorticoids:</u> do not have specific plasma transport protein.**

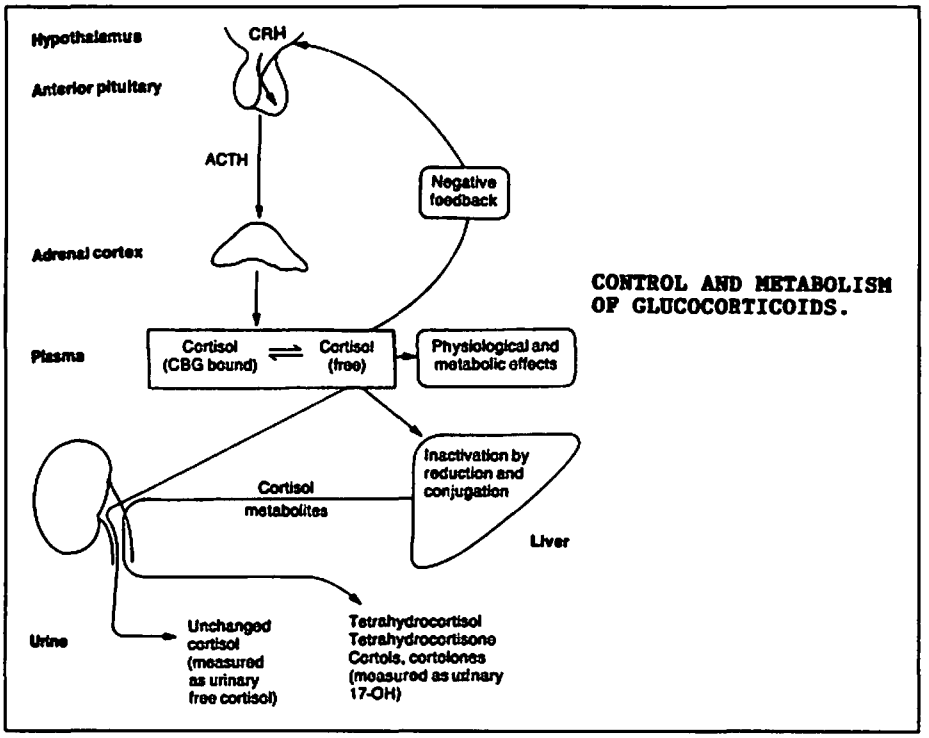
IV. *REGULATION (CONTROL) OF SECRETION:*

A. <u>Glucocorticoids</u>:

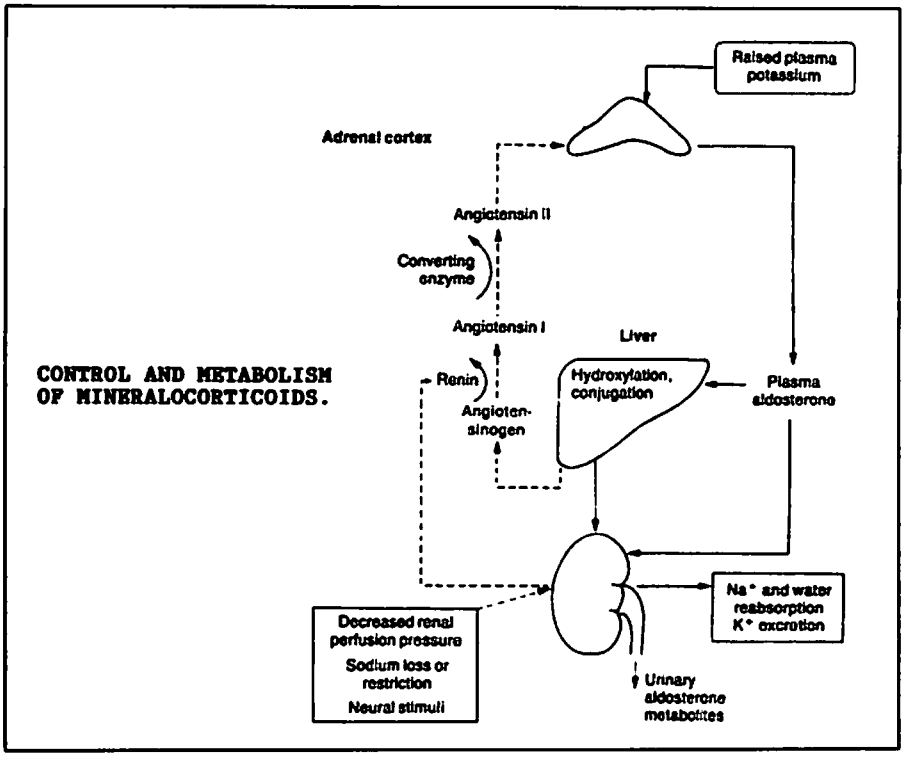
1. **When the body is stressed, corticotropin releasing hormone (CRH) is released by the hypothalamus, which stimulates anterior pituitary to produce ACTH.**
2. **ACTH binds with receptors in the cell membrane of zona fasiculata and zona reticularis. This leads to activation of adenylate cyclase and conversion of ATP into cyclic AMP.**
3. **Cyclic AMP will stimulate formation and secretion of glucocorticoids.**

B. <u>Mineralocorticoids:</u>

1. **When the body is subjected to hpotension, (decreased renal perfusion pressure), anoxia or kidney trauma, the kidney responds by secreting renin hormone.**
2. **Renin will activate angiotensinogen into angiotensin I and II.**
3. **Angiotinsin II will stimulate zona retucularis to produce mineralocorticoids (aldosterone) which aact on distal convoluted tubules of the kidney causing Na^+ and water reabsorption and K^+ excretion.**



CONTROL AND METABOLISM OF GLUCOCORTICOIDS.



V. *METABOLISM AND EXCRETION:*

A. Steroid hormones are metabolized in the liver where they are reduced and conjugated with either glucuronic acid or sulphate.
   1. About 20 % to 30 % of those metabolites are secreted into the bile and then excreted in the feces.
   2. The reminder are released into the blood to be excreted by the kidney with urine. These conjugate metabolites are soluble in blood or bile and need no carrier proteins.

VI. *FUNCTIONS (EFFECTS of CORTICAL HORMONES):*

B. <u>Glucocorticoids:</u> See the following table.

C. <u>Mineralocorticoids</u>:
   1. The most active member of these hormones is the aldosterone.
   2. They act in the kidney to stimulate transport by the distal convoluted tubules and collecting tubules, leading to Na^+ retention.
   3. These hormones also promote the secretion of K^+, H^+ and NH_4^+ by the kidney.

VII. *Hyper and hypofunction of glucocorticoids and mineralocorticoids:*

A. <u>Overproduction of glucocorticoids (Cushing`s syndrome)</u>: Discussed before in ACTH.

B. <u>Underproduction of glucocorticoids (Addison`s syndrome)</u>:

1. It results in hypoglycemia, anoroxia, weight loss, intolerance to stress and severe weakness.

C. Overproduction of mineralocorticoids (Conn`s syndrome):

1. The classical manifestations include hypertension, hypokalemia, hypernatremia and alkalosis.

I. ***EFFECTS ON INTERMEDIARY METABOLISM:***

A. Increase glucose production in liver by stimulating gluconeogenesis (stimulate synthesis of enzymes of gluconeogesis and increase the delivary of amino acids (the gluconeogenic substrate) from peripheral tissues).

B. Increase hepatic glycogen deposition by promting the activation of glycogen synthetase.

C. Promote lipolysis (in extremities) but can cause lipogenesis in other sites (face and trunk) especialy at higher than physiologic levels.

D. Promote protein and RNA metabolism. This is an anabolic effect at physiologic levels, but can be catabolic in certain conditions and at higher than physiologic levels.

II. ***EFFECTS ON HOST DEFENSE MECHANISMS:***

A. Suppress the immune responce.

B. Suppress the inflammatory response by:

1. Decreasing the number of circulating leukocytes and the migration of tissue leukocytes .
2. Inhibiting fibroblast proliferation.

III. ***OTHER EFFECTS:***

A. Necessary for maintenance of normal blood pressure and cardiac output.

B. Required for maintenance of normal water and electrolyte balance. Perhaps by restraining ADH release (H_2O) and by increasing angiotensinogen (Na^+). These effects contribute to the effect on blood pressure.

C. Necessary, with the hormones of the adrenal medulla, in allowing the organism to respond to stress.

The diverse effects of glucocorticoids.

Hormones of adrenal medulla

They include catecholamine hormones: epinephrine, norepinephrine and dopamine (see protein metabolism).

Hormones of gonads

I. The gonads have 2 functions which are production of germ cells and sex hormones.

A. In males: testes produce spermatozoa and testosterone.

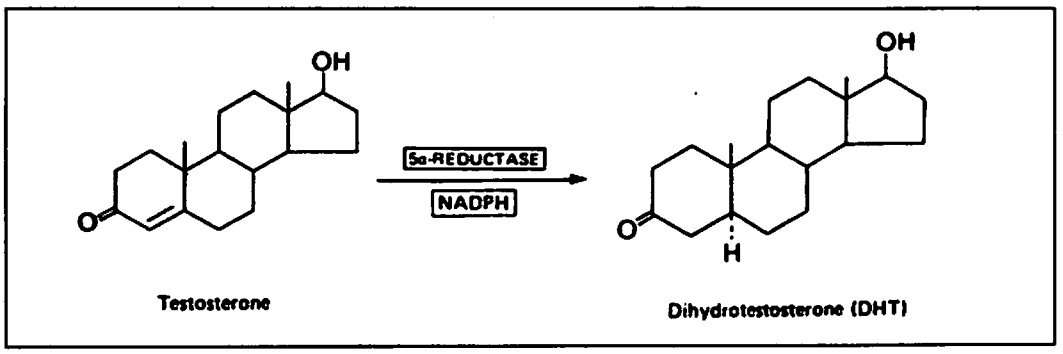
B. **In females:** Ovaries produce ova and the steroid hormones estrogens and progesterone.

II. *Male sex hormones (androgens):*

A. Androgens are produced by the **Leydig cells** and the **sertoli cells** of the testes.

B. Ovaries produce also androgens in small amounts.

C. Many androgens are produced by the **testes**, but the most active members are **testosterone** and its metabolite **dihydrotestosterone (DHT)**.



D. Sertoli cells also produce an **androgen binding protein (ABP)** which binds tostosterone and dihydrotestosterone . ABP is secreted into the lumen of semineferous tubules and in this position , it binds testosterone (produced by Leydig cells) and transports it in very high concentration to the site of spermatogenesis . This explains why testosterone when given as a drug does not support spermatogenesis.

E. **Biosynthesis of androgens:**

1. Testosterone is synthesized from cholesterol by a pathway similar to that described for steroidogenesis in adrenal cortex.

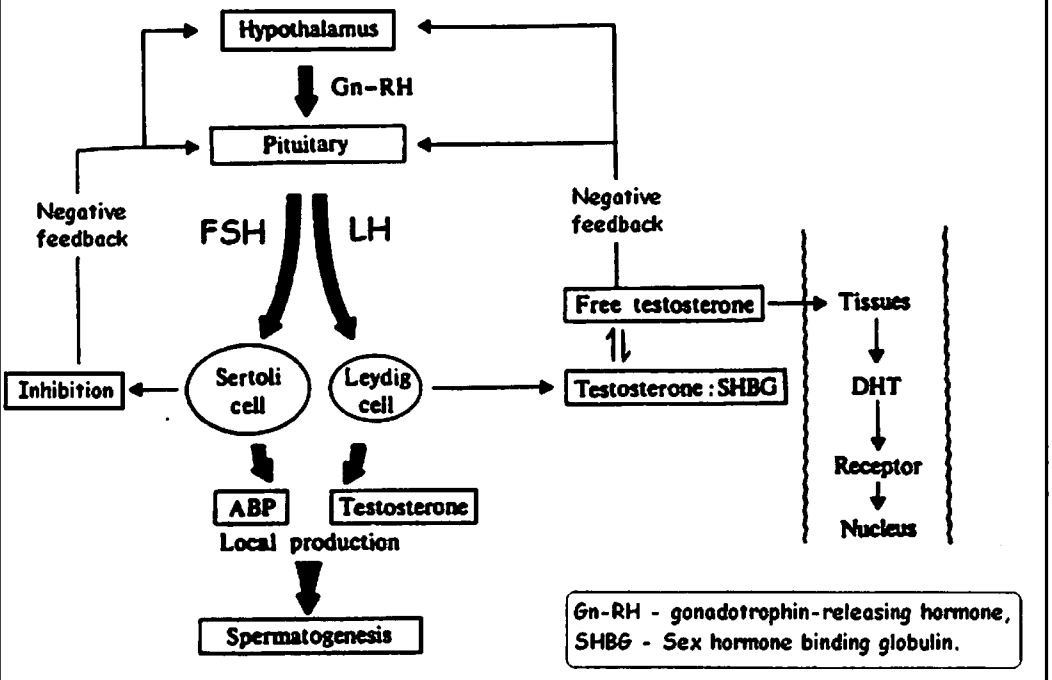
F. **Plasma transport of testosterone:**

1. Testosterone and DHT circulate in plasma in free form (2%) and in association with protein(98%).
2. The free testosterone is the biologically active form of hormone.
3. The testosterone binding protein is called: testosterone-estrogen-binding globulin (TEBG) or sex hormone binding globulin (SHBG) which is produced by the liver.

G. **Regulation (control) of secretion:**

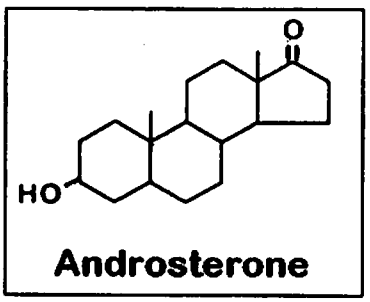
1. LH stimulates steroidogenesis and testosterone production by binding to receptors on the cell membrane of the Leydig cells (by a machanism similar to that of ACTH in the adrenal cortex).
2. FSH binds to the sertoli cells and promotes the synthesis of androgen-binding protein (ABP) which binds testosterone and

secreted in the lumen of semineferous tubules. This will stimulate spermatogenesis.



The hypothalamic - pituitary-testicular axis.

H. **Metabolism and excretion:**



1. The metabolic and products of testosterone is 17-ketosteroids which contain ketone group at C-17. The most important member is androsterone.
2. Testosterone is metabolized in liver, its metabolites conjugate with sulfate or glucuronate before excretion in urine.
3. 17-ketosteroids are also end products of androgens of adrenal cortex.

I. **Functions of androgens:**

1) The androgens, mainly testosterone and DHT are involved in the following functions:

2. Sexual differentiation (male or female).
3. Spermatogenesis .
4. Development of secondary sex characters e.g. male voice, male pattern of hair distribution.
5. Anabolic metabolism and gene regulation.

a) There is an effect on brain, leading to characteristic male sexual behaviour and aggressiveness.

J. **Hypogonadism:**

1. This is a condition of deficiency of testosterone synthesis .
2. It may be due to:
   a) Primary hypogonadism: due to absence or disease of the testes.
   b) Secondary hypogonadism: due to defective secretion of LH &/ or FSH .
3. It is characterized by impotence, obesity and molecular wasting (due to loss of the protein anabolising effect of testosterone).

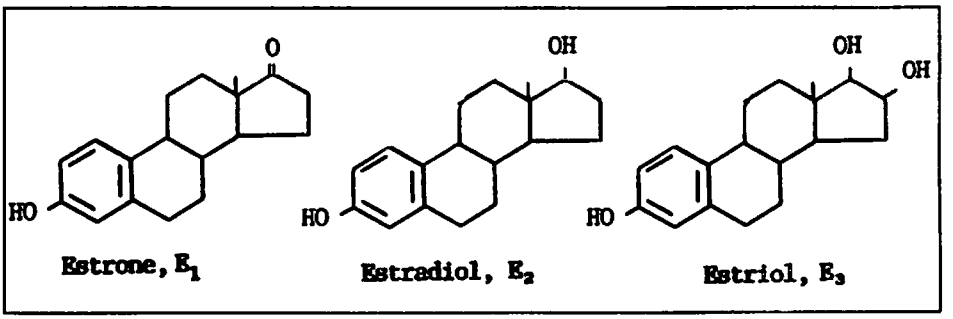
III. *FEMALE SEX HORMONES (ESTROGENS AND PROGESTINS):*

A. **Estrogens are produced by:**

1. Follicles and corpus luteum of the ovary.
2. Placenta which produces increased amount in the second and third trimesters of pregnancy.

B. **Three types of estrogens are present:**

1. Estradiol (E_2) and estrone (E_1) are produced by the ovary.
2. Estriol (E_3) is produced by the placenta.

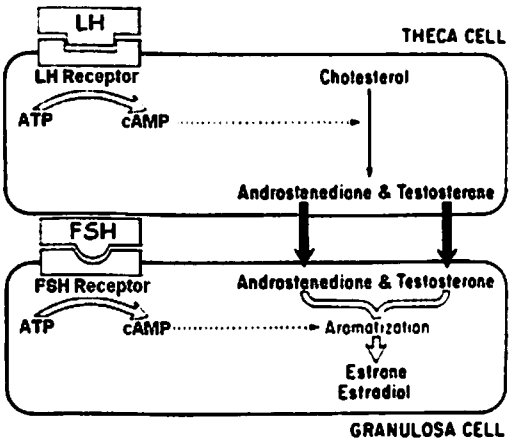


Estrone, E_1 Estradiol, E_2 Estriol, E_3

C. Progestins (progesterone) are produced and secreted by corpus luteum.

D. **Biosynthesis of estrogens and progesterone:**

1. It is similar to those of male hormones.
2. Estrogens are formed by the aromatization of androgens in a complex process that involves 3 hydroxylation steps by theca and granulosa cells of the ovaries.



"Two-cell hypothesis" of ovarian estrogen production. Luteinizing hormone stimulates androgen production in thecal cells. Androgens then diffuse into granulosa layer, where they are aromatized into estrogens, in an action specifically stimulated by FSH.

3. **There is two cell hypothesis presents an explanation of ovarian steroidogenesis. It proposes that LH acts on the theca cells of the ovary to produce androgens. Those androgens are transferred from theca cells into granulosa cells where before ovulation, under the effect of FSH the androgens are converted to estrogens by the enzyme aromatase. After ovulation the granulosa cells secrete estrogens and progesterone directly into the blood stream under the influence of FSH.**

E. <u>Plasma transport of estrogens and progesterone</u>:

1. **Estrogens are bound to testosterone-estrogen binding globulin (TEBG), and progesterone is bound to corticosteroid binding globulin (CBG).**
2. **Only the free (unbound) hormones have biological activity.**

F. <u>Regulation (control) of secretion</u>:

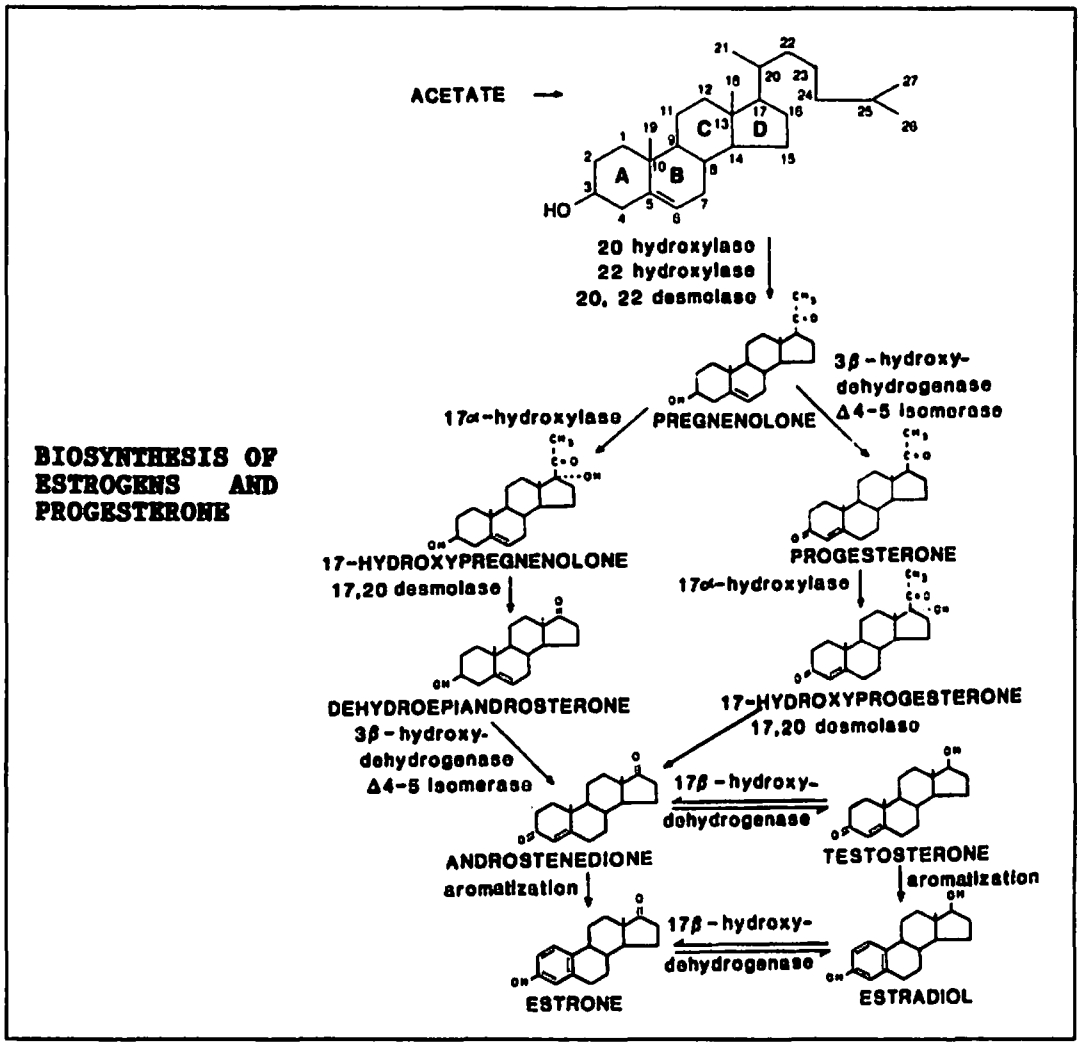
1. **As mentioned before combined action of FSH and LH is required for the synthesis of estradiol.**
2. **LH is required for ovulation and synthesis of progesterone.**

G. <u>Metabolism and excretion</u>:

1. **Estrogens : The liver converts estradiol and estrone to estriol which conjugates with sulfate or glucoronic acid before excreted in urine or bile.**
2. **Progesterone: The liver converts progesterone into a compound called pregnandiol which conjugates with sulfate or glucoronic acid and excreted in urine.**

H. <u>Functions</u>:

1. **Estrogens :**
   - ***a)*** **Estrogens stimulate the growth of the cells of uterus, vagina, graafian follicles of the ovary and the mammary gland.**
   - ***b)*** **Estrogens are responsible for the development of secondary sexual characters e.g. female voice, female pattern of hair and fat distribution.**
   - ***c)*** **Estrogens induce, in the uterus and mammary gland , the synthesis of progesterone receptors.**
   - ***d)*** **Estrogens are responsible for the maintenance of the menstrual cycle.**
   - ***e)*** **Estrogens are required for the development of mammary gland.**



2. **Progesterone :**
   - *a)* **Progesterone is necessary for the implantation of fertilized ovum in the uterus.**
   - *b)* **Progesterone inhibits uterine contraction during pregnancy (i.e. maintain pregnancy).**
   - *c)* **Progesterone stimulates the growth of the secretory glands of uterus and mammary gland.**
   - *d)* **Progesterone is responsible for the maintenance of menstrual cycle (luteal phase).**
   - *e)* **Progesterone antagonizes the action of estrogens in various tissues.**

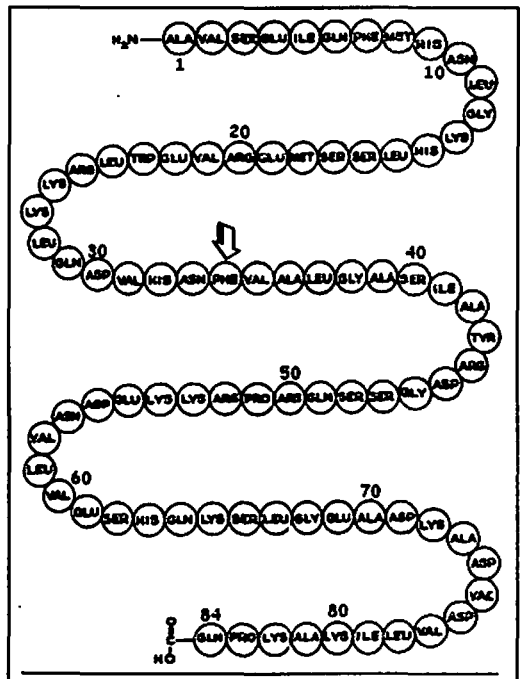
Hormones that regulate calcium metabolism

These hormones are involved in the regulation of blood calcium: parathyroid hormone (PTH), calcitriol and calcitonin.

I. Parathyroid hormone:

A. Structure and synthesis:

1. It is polypeptide chain of 84 amino acids and molecular weight 9500.
2. The physiological activity of the hormone on both skeleton and renal tissues are contained within the 34 amino acids counting from the amino terminal end (- NH_2) of the molecule.
3. PTH is synthesized in parathyroid gland as precursor of 115 amino acids called prepro PTH. A 25 amino acids peptide is cleaved at N-terminal giving proPTH of 90 amino acids. 6 Amino acids peptide is then removed from proPTH to give mature PTH.



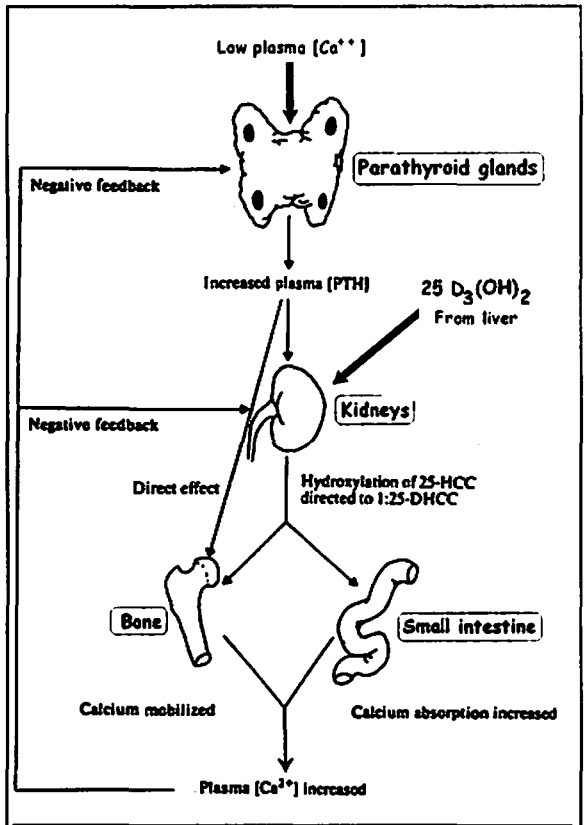
Parathyroid hormone.

B. Regulation of secretion:

1. PTH secretion is inversely related to the concentration of ionized calcium. Low blood
2. ionized calcium stimulates PTH secretion and vice versa.

C. Mechanism of action of PTH:

1. PTH binds to a receptor in a cell membrane of bone and kidney cells.
2. Its second messenger inside target cells are calcium and cyclic AMP.



Regulation of PTH secretion.

D. Functions of PTH:

1. Maintenance of plasma calcium level within normal level by:
   a) Formation of calcitriol essential for intestinal calcium absorption .

b) **Mobilization of calcium (Ca^{++}) from bones .**

c) **Reduction of renal calcium excretion .**

2. **PTH increases the urinary excretion of phosphate .**

E. <u>Hypoparathyroidism</u>:

1. **It is a condition resulting from insufficient amount of PTH.**
2. **It is characterized by decreased serum ionized calcium and elevated serum phosphate.**
3. **Symptoms include neuromuscular irritability , tetany and in severe cases tetanic paralysis of respiratory muscles ➔ Death.**
4. **Causes:**

a) **Primary hypoparathyroidism: due to autoimmune destruction of the gland (rare).**

b) **Secondary hypoparathyroidism : more common, It is due to accidental removal or damage of the glands during neck surgery e.g. thyroidectomy.**

F. <u>Hyperparathyroidism</u>:

1. **It is a condition resulting from excessive production of PTH.**
2. **It is characterized by elevated serum ionized calcium and PTH and decreased serum phosphate levels.**
3. **Symptoms and signs: include extensive bone resorption, kidney stones formation and frequent urinary tract infections.**
4. **Causes:**

a) **Primary hyperparathyroidism: it is usually due to parathyroid adenoma, but may be due to parathyroid hyperplasia or ectopic production of PTH by a malignant tumour else where in the body.**

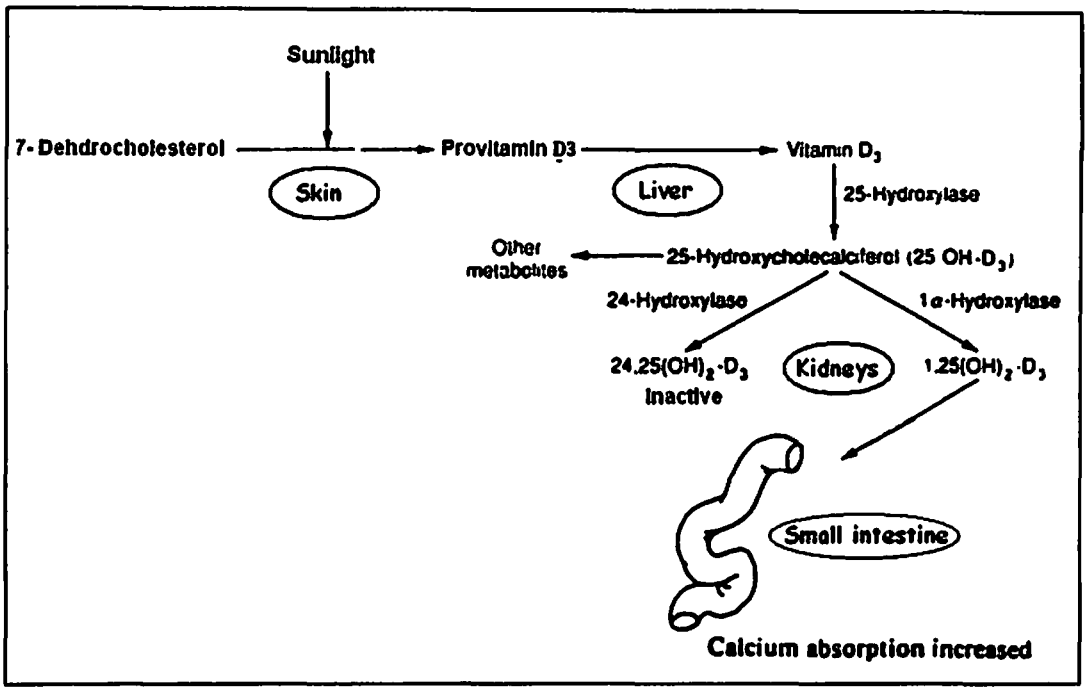
b) **Secondary hyperparathyroidism: due to renal failure which results in decreased conversion of 25 OH-D_3 to 1,25 $(OH)_2$-D_3. This leads to insufficient calcium absorption in the gut and secondary release of PTH in an attempt to maintain normal calcium levels .**

c) **Tertiary hyperparathyroidism: due to the development of functioning parathyroid adenoma as a complication of previously existing secondary hyperparathyroidism.**

II. *CALCITRIOL (1,25 DIHYDROXYCHOLECALCIFEROL):*

A. Structure and synthesis:

1. Discused in vitamin D part I.



B. Regulation of calcitriol synthesis: synthesis of calcitriol is regulated by :

1. **The level of plasma calcium: Hypocalcemia stimulates 1-hydroxylase enzyme present in the kidney which leads to calcitriol formation . This effect requires PTH, which is also released in response to hypocalemia.**
2. **Calcitriol itself is an important regulator of its own production. High levels of calcitriol inhibit renal 1-α hydroxylase.**

C. Mechanism of action of calcitriol: Calcitriol acts at the cellular level in a manner similar to other steroid hormones (see vitamin D, part I).

D. Functions of calcitriol:

1. **Normalization of plasma calcium :, through :**
   - ***a)*** **Increases of the intestinal absorption of calcium (by stimulating synthesis of specific mRNA responsible for the synthesis of calcium binding protein (calbindin) in intestinal mucosal cells).**
   - ***b)*** **Reabsorption of calcium from bones and kidney.**
2. **Mineralization of bones:**
   - ***a)*** **Calcitriol stimulates the synthesis of osteocalcin which is calcium binding protein present in bones.**

E. **Vitamin D deficiency**: It results in rickets in children and osteomalcia in adults (see vitamin D part I).

III. *CALCITONIN:*

A. Calcitonine is a 32-amino acid peptide secreted by the parafollicular C cells of the human thyroid.

B. Its function in regulation of plasma calcium in human is uncertain. However, it may have a role in decreasing plasma calcium by increasing deposition of calcium in bones.

Hormones of the pancreas and Gastrointestinal tract

I. ***Pancreas is 2 different organs*** contained with in one structure:

A. **Exocrine gland:** which is the acinar portion of the pancreas, secreting into the duodenal lumen the enzymes and ions used for digestion.

B. **Endocrine gland**: which is the islets of Langerhans.

II. There are 1-2 million islets in human pancreas make up 1-2 % of its weight, and they are 4 types, each secretes a specific hormone:

A. **A or alpha cells**: secrete glucagon.

B. **B or beta cells**: secrete insulin.

C. **D or delta cells**: secrete somatostatin.

D. **F cells**: secrete pancreatic polypeptide.

III. *INSULIN:*

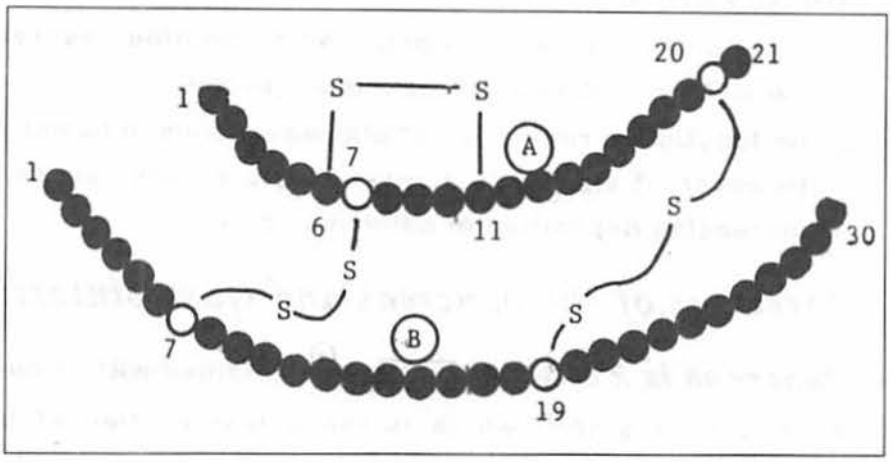
A. **Insulin: has an impressive list of "firsts". It was:**

1. The first protein proved to have hormonal action.
2. The first protein crystalized .
3. The first protein sequenced for its amino acids.
4. The first protein synthesized by chemical techniques.
5. The first protein shown to be synthesized as a large precursor molecule"preproinsulin".
6. The first protein prepared for commercial use by recombinate DNA technology.

B. **Structure:**

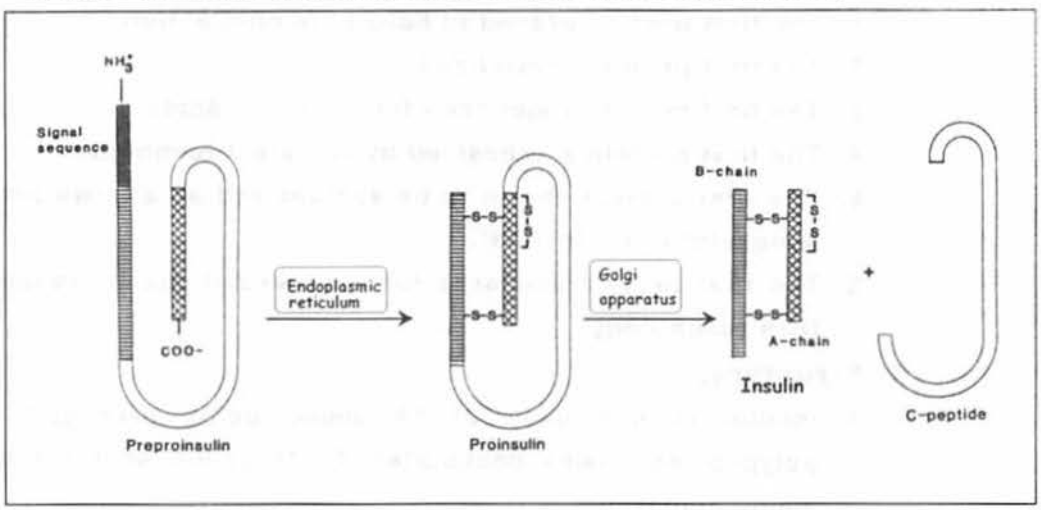
1. Insulin is composed of 51 amino acids arranged in two polypeptide chains designated A (21 amino acids) and B (30 amino acids).

2. A and B chains are linked together by two disulfide bridges [between amino acids 7 (A) & 7 (B) and 20 (A) & 19(B)]. The insulin molecule also contains an intra molecule disulfide bridge between amino acids 6 and 11 of the A chain.



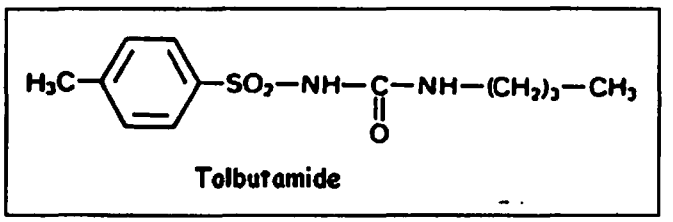
C. Boisynthesis:

1. Insulin is synthesized as long polypeptide of 110 amino acids called :**preproinsulin.**
2. 24 Amino acid residues "**signal sequence**" at the N-terminal end of preproinsulin are cleaved in **endoplasmic reticulum** to form another precursor called **proinsulin** (86 amino acids).
3. The proinsulin is transferred to the **Golgi apparatus**, where it under goes protolysis to give **Insulin** and **c-peptide.**
4. In Golgi apparatus also insulin combines with **zinc** and packed into **secretory granules**. Zinc is essential for insulin activity.
5. Both insulin and c-peptide are secreted into the circulation in equimolar amounts.
6. Approximately 50 units of insulin per day are required; this is about 1/5 of the amount stored in the human pancreas.



D. Regulation of insulin secretion:

1. **Stimulation of insulin secretion :Insulin secretion by the β-cells of the islets of Langerhans is closely coordinated with the release of glucagon by the pancreatic α-cells. The relative amounts of insulin and glucagon released by the pancreas are regulated so that: the rate of hepatic glucose production is kept equal to the amount of glucose used by the peripheral tissues. The following substances stimulate and increase :insulin secretion:**
   - ***a)*** **Glucose :An increase in plasma glucose concentration is the most important stimulus of insulin secretion. At the same time glucose inhibits glucagon release.**
   - ***b)*** **Hormones :Growth hormone, cortisol, estrogens, progesterone and placental lactogen.**
   - ***c)*** **Gastrointestinal hormones: as secretion.**
   - ***d)*** **Amino acids :after ingestion of protein.**
   - ***e)*** **Pharmacological drugs: many drugs stimulate insulin secretion and used in treatment of diabetes mellitus especially type II. These drugs are: sulphonylurea compounds and tolbutamide.**

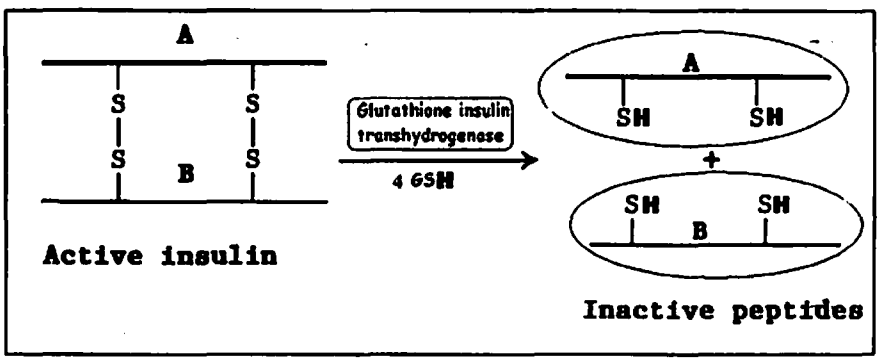


Tolbutamide

2. **Inhibition of insulin secretion :**
   Epinephrine: inhibits insulin secretion even in the presence of glucose.

E. Insulin degradation: Insulin-after carrying out its physiologic functions-is broken down by 2 enzymes:

1. **Protease enzyme: which is present in many tissues.**
2. **Glutathione insulin transhydrogenase: This enzyme reduces the disulfide bonds of insulin, separating it into 2 separate inactive polypeptides. This reaction needs glutathione as a reducing substance.**



Active insulin

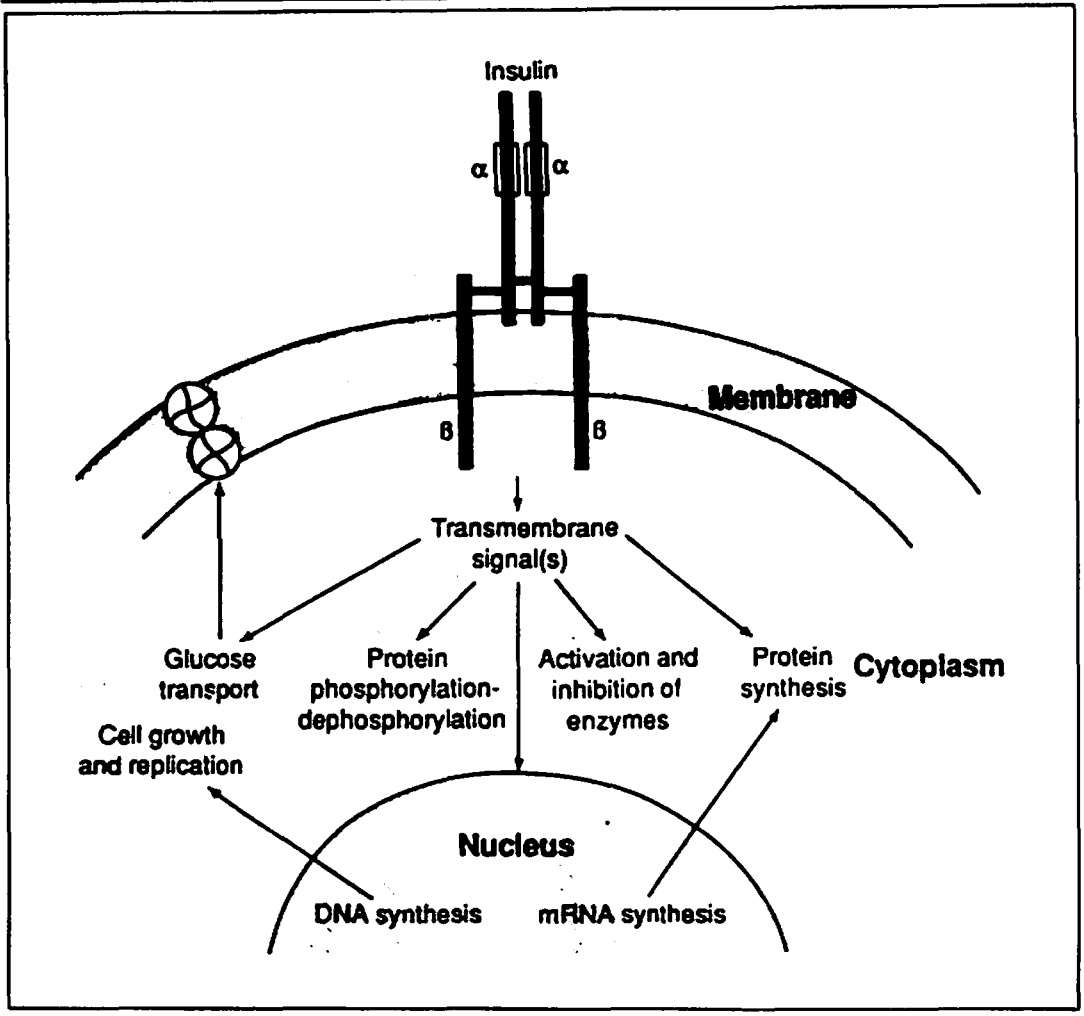
Inactive peptides

F. Functions (metabolic effects) of insulin:

1. **Effects on carbohydrate metabolism:** The intravenous administration of insulin causes an immediate decrease in the concentration of blood glucose. This decrease is caused by:
   - *a)* **Increased glucose transport into cells** (except for liver, brain, lens , intestinal mucosa, renal tubules and red blood cells where glucose transport does not depend on insulin but passes freely).
   - *b)* **Increased glycogen synthesis** (glycogenesis).
   - *c)* **Decraese gluconeogenesis.**
2. **Effects on lipid metabolism:**
   - *a)* **Inhibition of lipolysis:** By inhibiting hormone sensitive triacylglycerol lipase in adipose tissue.
   - *b)* **Stimulation of lipogenesis:** Insulin increases the transport of glucose, providing the substances (glycerol-phosphate, acetyl CoA and NADPH) needed for fatty acid synthesis, and hence lipogenesis.
3. **Effect on protein synthesis:** Insulin stimulates the entery of amino acids into cells and protein synthesis in most tissues.
4. **effect on cell replication:**
   - *a)* Insulin has effects on **cell growth and replication** .
   - *b)* It has also effects on **fetal organogenesis** and **differentiation** and in tissue repair and regeneration.

G. Mechanism of insulin action:

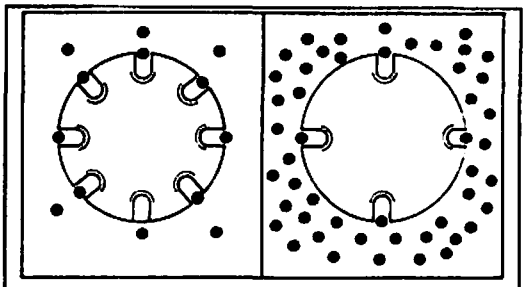
1. **Insulin receptors:**
   - *a)* Insulin binds to specific, high affinity receptors in the cell membrane of most tissues.
   - *b)* After binding to the cell surface, the insulin-receptor complex enters the cell by pinocytosis, producing a membrane-bound vesicle.
   - *c)* Insulin then carries out many reactions (through unknown second messenger) which finally leads to a diverse biological effects, on carbohydrate, protein, lipids and cell replication.
   - *d)* The insulin is ultimately degraded in the lysosomes and the receptors return to the cell membrane.
2. **Membrane effect of insulin:** Insulin, once bound to the receptor, causes an increase on the transport of glucose across the cell membrane.



3. **Intracellular effects of insulin: The binding of insulin to its receptor stimulates a series of reactions that lead to the activation or inhibition of key enzymes in the cell through:**
   - ***a)*** **Activation and inhibition of enzymes: e.g. insulin activates phosphodiesterase enzyme that converts cAMP into AMP (see part II, glycogen metabolism and lipogenesis).**
   - ***b)*** **Phosphorylation and dephosphorylation of enzymes: e.g. insulin stimulates phosphoprotein phosphatase enzymes that dephosphorylate glycogen synthase (activating it) and glycogen phosphorylase (inactivating it). (see part II, glycogen metabolism and lipolysis).**
   - ***c)*** **Stimulation of translation of mRNA for at least 50 proteins in a variety of tissues.**
   - ***d)*** **Regulation of transcription of mRNA e.g. insulin inhibits transcription of mRNA of phosphoenol pyruvate carboxykinase enzyme, leading to inhibition of gluconeogenesis.**

H. **Down regulation of insulin receptors:**

1. Insulin receptors are found on most mammalian cells, in cocentration up to 20,000 per cell.
2. In conditions in which plasma insulin levels are high e.g. obesity or acromegaly the number of insulin receptors is decreased and target tissues become less sesitive to insulin.



Down regulation: left, 8 insulin receptors with near normal concentration of insulin. right, 4 insulin receptors with high insulin concentration.

3. This decrease is called: "down regulation" and it results from the loss of receptors by internalization.
4. Down regulation explains part of insulin resistance in obesity and type II diabetes mellitus.

I. **Diabetes mellitus:**

1. This is a disease resulting from a deficiency of insulin action (due to absence of, or resistance to insulin). It is discussed before in carbohydrate metabolism part II.

IV. *GLUCAGON:*

A. Glucagon is a polypeptide hormone secreted primerily by the α-cells of the pancreatic islets. Glucagon together with epinephrine, cortisol and growth hormone oppose many of the actions of insulin.

B. **Structure of glucagon:** Glucagon is composed of 29 amino acids arranged in a single polypeptide chain.

C. **Biosynthesis of glucagon:** It is synthesized as a large precursor molecule "preproglucagon" that is converted to glucagon through a series of selective proteolytic cleavages, similar to those described for insulin biosynthesis.

D. **Regulation of glucagon secretion:**

1. **Stimulation of secretion:** glucagon secretion is stimulated by low blood glucose, amino acids and epinephrine.
2. **Inhibition of secretion:** glucagon secretion is inhibited by increased blood sugar. This occurs after ingestion of carbohydrate-rich meal.

E. **Functions (metabolic effects)of glucagon:**

1. **Effects on carbohydrate metabolism:** It increases blood glucose level through:

a) **Stimulation of glycogenolysis (in liver and not in muscles).**

b) **Stimulation of gluconeogenesis.**

2. **Effects on lipid metabolism:**
   a) **Glucagon helps the oxidation of fatty acids in liver and subsequent formation of ketones from acetyl CoA.**
   b) **Glucagon also stimulates lipolysis through stimulation of hormone-sensitive triacylglycerol lipase.**
3. **Effects on protein metabolism:**
   a) **Glucagon increases the uptake of amino acids by the liver, for gluconeogenesis.**
4. **Mechanism of action of glucagon:**
   a) **Glucagon binds to a membrane receptor of target cells such as liver and adipose tissue.**
   b) **This will stimulate adenylate cyclase enzymes in the cell membrane , whch in turn causes a rise in cAMP (the second messenger). cAMP activates protein kinases and increases the phosphorylation of specific enzymes e.g. glycogen synthase and phosphorylase.**

V. *SOMATOSTATIN:*

A. It is 14-amino acid peptide which is synthesized in D-cells of the pancreatic islets.

B. In addition to its presence in pancreatic islets, somatostatin is found in the hypothalamus and many gastrointestinal tissues.

C. <u>Functions</u>:

1. **Pancreatic somatostatin :Inhibits the release of other islets cell hormones: insulin, glucagon and pancreatic polypeptide.**
2. **Hypothalamic somatostatin: Inhibits the release of growth hormone, TSH, FSH and ACTH.**
3. **Gastrointestinal somatostatin: Inhibits the absorption of nutrients because:**
   a) **It prolongs gastric emptying.**
   b) **It decreases gastric secretion and gastric acid production.**
   c) **It decreases the digestive enzymes secreted by pancreas.**
   d) **It decreases visceral blood flow.**
   e) **It slows sugar absorption.**

VI. *PANCREATIC POLYPEPTIDE:*

A. It is 36-amino acid peptide secreted by F-cells of pancreatic islets.

B. Its function is unknown but it may haves effects on hepatic glycogen and gastrointstinal secretion.

VII. *GASTROINTESTINAL HORMONES:*

Hormone	Location	Major action
Gastrin	Gastric antrum, duodenum	Gastric acid and pepsin secretion
Cholecystokinin (CCK)	Duodenum, jejunum	Pancreatic amylase secretion
Secretin	Duodenum, jejunum	Pancreatic bicarbonate secretion
Gastric inhibitory polypeptide (GIP)	Small bowel	Enhances glucose-mediated insulin release; inhibits gastric acid secretion
Vasoactive intestinal polypeptide (VIP)	Pancreas	Smooth muscle relaxation; stimulates pancreatic bicarbonate secretion
Motilin	Small bowel	Initiates interdigestive intestinal motility
Somatostatin	Stomach, duodenum, pancreas	Numerous inhibitory effects
Pancreatic polypeptide (PP)	Pancreas	Inhibits pancreatic bicarbonate and protein secretion
Enkephalins	Stomach, duodenum, gallbladder	Opiate-like actions
Substance P	Entire gastrointestinal tract	Physiologic actions uncertain
Bombesin-like immunoreactivity (BLI)	Stomach, duodenum	Stimulates release of gastrin and CCK
Neurotensin	Ileum	Physiologic actions unknown
Enteroglucagon	Pancreas, small intestine	Physiologic actions unknown

Hormones of thyroid gland

See protein metabolism, part III

Hormones of the kidney

I. In addition to excretory functions, the kidney acts as an endocrine gland, secreting many hormones.

II. Kidney hormones can be classified into:

A. Hormones acting on blood vessels:

1. Hormones cause vasoconstriction:

a) Renin.

2. **Hormones cause vasodilatation:**
   - *a)* **Renomedullary prostaglandins.**
   - *b)* **Antihypertensive renomedullary lipids.**
   - *c)* **Kininogen.**

B. Hormones acting for RBCs production (Erythropoiesis):

1. **Erythropoietin.**
2. **Erythrogenin.**

C. Hormones acting for calcium metabolism: by increasing intestinal calcium absorption and its deposition in bones.

1. **Calcitriol [1,25 $(OH)_2$ - D_3].**
2. **24,25 $(OH)_2$ - D_3.**

Eicosanoids and related compounds

See lipid metabolism part II.

Summary of major endocrine hoemones :

Hormones	Target tissues	Chemical nature	Primary actions
Steroid hormones			
Androgens (male sex hormones) .Testosterone (and other androgens)	Peripheral tissues, testis.	Steroid	Stimulates development of secodary sex characters in men, protein anabolic effect.
Estrogens(female sex hormones), Esterone, estradiol	Peripheral tissues, ovary	Stroid	Stmulates development of secondary sex charteristics in women , protein anabolic effect .
Progesterone	Uterus	Steroid	Prepares uterus for implantaion of ovum.
Adrenal cortex hormones			
11-Deoxycortisol	Renal distal tubule, large intestine.	Steroid	Maintains electrolyte and water balance
Cortisone or hydrocortisone (cortisol)	Muscle, liver, adipose cells	Steroids	Stimulates gluconeogenesis from amino acids,anti-insulin effects on glucose and fat metabolism
Aldosterone	Renal distal tubule, large intesines	Steroid	Regulates retention of sodium ions, excretion of potassium ions
Amino acid-derived hormones			
Adrenal medulla hormones (Epinephrine Norepinephrine)	Liver, adipose cells	Phenolic amines	Stimulates glycogenolysis, hyprtensive effect .
Thyroid hormones.	Most tissues	Amino acid (iodinated) thyroxine or derivatives	Regulates rate of metabolism , increases serum glucose.
Protein and polypeptide hormones			
Anterior pituitary, thyroid stimulating hormone (TSH), thyrotropin	Thyroid	Protein	Stimulates development and secretion of thyroid gland.
Adrenocorticotropic hormone(ACTH)	Adrenal cortex	protein	Stimulates growth and secretion of adrenal cortex
Follicle-stimulating hormone(FSH)	Ovarian follicle (women), seminephrous tubules (men)	protein	Stimulates growth of follicles and production of estrogen in women , formation of spermatozoa in men.
Luteinizing hormone	Corpus luteum	protein	Triggers formation of corpus

(LH)	(women), testes (men)		luteum and production of progestrone in women , production of androgens by interstatial cells in men.
Prolactin (lactogenic hormone, LTH).	Milk glands	protein	Initiates lactation
Growth hormone (GH)	Most tissues	protein	Stimulates growth (also affects fat and carbohydrate metabolism .
Posterior pituitary hormones			
Vasopressin, (antidiuretic hormone, (ADH)	Renal collecting ducts, bladder	peptide	Stimulates reabsorption of water in kidney tubule
Oxytocin (Pictocin)	Uterus, mammary glands	peptide	Contracts uterus
Calcitonin (thyroid gland).	Bone	peptide	Lowers serum calcium
Parathyroid hormone	Bone,small intestine, kidney	protein	Regulates blood calcium
Pancreatic hormones			
Insulin	Most cells	peptide	Facilitates carbohydrate catabolism
Glucagon	Liver	peptide	Raises blood glucose by hepatic glycogenolysis .
Gastrointestinal hormones			
Secretin	Pancreas, gallbladder.	peptide	Stimulates flow of pancreatic juice and bile to a much smaller extent
Cholecystokinin-pancreozymin	Gallbladder pancreas	peptide	Contraction of gallbladder; stimulates secretion of pancreatic enzymes.
Gastrin	Stomach	peptide	Stimulates secretion of gastric juice (HCl)
Renal erythropoietin	Bone	peptide	Stimulates red cell formation
Hypothalamic factors	Anterior pituitary	peptides	Stimulates or inhibits release of corresponding trophic hormones.

Commercial Press - Kalyoub - Egypt.

Biochemistry

This book "Oraby's Biochemistry " by SAID ORABY is made in it's four parts (I, II, III and IV) to provide necessary knowledge and recent information about biochemistry for medical students and allied sciences.

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- **Latest advances in biochemistry important to medicine.**
- **Many illustrations are added to bring biochemistry alive.**
- **Part IV (questions and answers): is a new part (2 volumes) to practice your studying and is the key to success.**
- **Postgraduates and students who are preparing for standard courses or examinations (fellowships, ECFMG.. etc) will find this book of benefit for them.**
- **Finally, I hope this work is appreciated and accepted by students and colleagues.**

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